

**MIXTURES OF DRUG-OLIGOMER
CONJUGATES COMPRISING POLYALKYLENE GLYCOL,
USES THEREOF, AND METHODS OF MAKING SAME**

Field Of The Invention

The present invention relates to drug-oligomer conjugates.

Background Of The Invention

Pharmaceutically active molecules such as proteins and polypeptides have been conjugated with polydispersed mixtures of polyethylene glycol or polydispersed mixtures of polyethylene glycol containing polymers to provide polydispersed mixtures of drug-oligomer conjugates. For example, U.S. Patent No. 4,179,337 to Davis et al. proposes conjugating polypeptides such as insulin with various polyethylene glycols such as MPEG-1900 and MPEG-5000 supplied by Union Carbide.

U.S. Patent No. 5,567,422 to Greenwald proposes the conjugation of biologically active nucleophiles with polyethylene glycols such as m-PEG-OH (Union Carbide), which has a number average molecular weight of 5,000 Daltons.

U.S. Patent No. 5,405,877 to Greenwald et al. proposes reacting bovine hemoglobin with thiazolidine thione activated PEG, which was prepared using m-PEG carboxylic acid having a number average molecular weight of 5,000 Daltons.

U.S. Patent No. 5,359,030 to Ekwuribe proposes conjugating polypeptides such as insulin with polyethylene glycol modified glycolipid polymers and polyethylene glycol modified fatty acid polymers. In this patent, the number average molecular weight of polymer resulting from each combination is preferred to be in the range of from about 500 to about 10,000 Daltons.

PEG is typically produced by base-catalyzed ring-opening polymerization of ethylene oxide. The reaction is initiated by adding ethylene oxide to ethylene glycol, with potassium

hydroxide as catalyst. This process results in a polydispersed mixture of polyethylene glycol polymers having a number average molecular weight within a given range of molecular weights. For example, PEG products offered by Sigma-Aldrich of Milwaukee, Wisconsin are provided in polydispersed mixtures such as PEG 400 (M_n 380-420); PEG 1,000 (M_n 950-
5 1,050); PEG 1,500 (M_n 1,400-1,600); and PEG 2,000 (M_n 1,900-2,200).

It is desirable to provide non-polydispersed mixtures of drug-oligomer conjugates that comprises polyalkylene glycol.

Summary Of The Invention

A mixture of drug-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may exhibit higher *in vivo* activity than a polydispersed mixture of similar conjugates, where the polydispersed mixture has the same number average molecular weight as the mixture according to the present invention. This heightened activity may result in lower dosage requirements. Moreover, a mixture of drug-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may be more effective at surviving an *in vitro* model of intestinal digestion than polydispersed mixtures of similar conjugates. Furthermore, a mixture of drug-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may result in less inter-subject variability than polydispersed mixtures of similar conjugates.

According to embodiments of the present invention, a substantially monodispersed mixture of conjugates each comprising a drug coupled to an oligomer that comprises a polyalkylene glycol moiety is provided. The mixture preferably is a monodispersed mixture and, more preferably, is a purely monodispersed mixture. The polyalkylene glycol moiety preferably has at least 2, 3 or 4 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety preferably has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety is preferably a polyethylene glycol moiety or polypropylene glycol moiety. The oligomer preferably further comprises a lipophilic moiety. The conjugate is preferably amphiphilically balanced such that the conjugate is aqueously soluble and able to penetrate biological membranes. The oligomer may comprise a first polyalkylene glycol moiety covalently coupled to the drug by a non-hydrolyzable bond and a second polyalkylene glycol moiety covalently coupled to the first polyalkylene glycol moiety by a hydrolyzable bond.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-
5 oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to still other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an *in*
10 *vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-
oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.
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According to yet other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.
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According to still other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a molecular weight distribution with a standard deviation of less than about 22 Daltons.
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According to yet other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a dispersity coefficient (DC) greater than
30 10,000 where

$$DC = \frac{\left(\sum_{i=1}^n NiMi \right)^2}{\sum_{i=1}^n NiMi^2 - \sum_{i=1}^n Ni - \left(\sum_{i=1}^n NiMi \right)^2}$$

wherein:

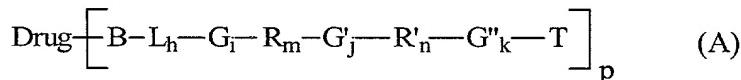
n is the number of different molecules in the sample;

N_i is the number of ith molecules in the sample; and

5 M_i is the mass of the ith molecule.

According to other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate includes a drug coupled to an oligomer and has the same number of polyalkylene glycol subunits.

According to still other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate has the same molecular weight and has the formula:



wherein:

B is a bonding moiety;

L is a linker moiety;

15 G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety;

20 h, i, j, k, m and n are individually 0 or 1, with the proviso that when R is the polyalkylene glycol moiety; m is 1, and when R' is the polyalkylene glycol moiety, n is 1; and

p is an integer from 1 to the number of nucleophilic residues on the drug.

Pharmaceutical compositions comprising conjugate mixtures of the present invention as well as methods of treating a disease state in a subject in need of such treatment by administering an effective amount of such pharmaceutical compositions are also provided.

25 Additionally, methods of synthesizing such conjugate mixtures are provided.

Drug-oligomer conjugate mixtures according to embodiments of the present invention may provide increased *in vivo* activity and/or lowered inter-subject variability and/or

decreased degradation by chymotrypsin when compared to conventional polydispersed drug-oligomer conjugate mixtures.

Brief Description of the Drawings

Figure 1 illustrates a generic scheme for synthesizing a mixture of activated polymers comprising a polyethylene glycol moiety and a fatty acid moiety according to embodiments of the present invention;

Figure 2 illustrates a scheme for synthesizing a mixture of mPEG according to embodiments of the present invention;

Figure 3 illustrates a scheme for synthesizing a mixture of activated mPEG7-hexyl oligomers according to embodiments of the present invention;

Figure 4 illustrates a scheme for synthesizing a mixture of activated mPEG7-octyl oligomers according to embodiments of the present invention;

Figure 5 illustrates a scheme for synthesizing a mixture of activated mPEG-decyl oligomers according to embodiments of the present invention;

Figure 6 illustrates a scheme for synthesizing a mixture of activated stearate-PEG6 oligomers according to embodiments of the present invention;

Figure 7 illustrates a scheme for synthesizing a mixture of activated stearate-PEG8 oligomers according to embodiments of the present invention;

Figure 8 illustrates a scheme for synthesizing a mixture of activated PEG3 oligomers according to embodiments of the present invention;

Figure 9 illustrates a scheme for synthesizing a mixture of activated palmitate-PEG3 oligomers according to embodiments of the present invention;

Figure 10 illustrates a scheme for synthesizing a mixture of activated PEG6 oligomers and conjugating human growth hormone with the activated PEG6 oligomers according to embodiments of the present invention;

Figure 11 illustrates a scheme for synthesizing various propylene glycol monomers according to embodiments of the present invention;

Figure 12 illustrates a scheme for synthesizing various propylene glycol polymers according to embodiments of the present invention;

Figure 13 illustrates a scheme for synthesizing various propylene glycol polymers according to embodiments of the present invention;

Figure 14 is an HPLC trace (HPLC gradient: 50% to 90% acetonitrile in 30 minutes) of the conjugation reaction illustrated in **Figure 10** using 2 equivalents of activated MPEG6 oligomers and 5 equivalents of activated MPEG6 oligomers;

5 **Figure 15** is an HPLC trace (HPLC gradient: 0% to 95% acetonitrile in 20 minutes) of the conjugation reaction illustrated in **Figure 10** using 30 equivalents of activated MPEG6 oligomers;

Figure 16 is a MALDI spectra of the conjugation reaction illustrated in **Figure 10** using 2 equivalents of activated MPEG6 oligomers;

10 **Figure 17** is an HPLC trace (HPLC gradient: 50% to 70% acetonitrile in 30 minutes) illustrating a partial purification of the product of the conjugation reaction of **Figure 10** using 5 equivalents of activated MPEG6 oligomers;

Figure 18 is a MALDI spectra of fraction B from the partial purification illustrated in **Figure 17**;

Figure 19 is a MALDI spectra of fraction C from the partial purification illustrated in **Figure 17**;

Figure 20 is a MALDI spectra of fractions D and E from the partial purification illustrated in **Figure 17**;

Figure 21 is an electrospray spectra of fraction E from the partial purification illustrated in **Figure 17**;

Figure 22 is an electrospray spectra of the reaction mixture from the conjugation reaction illustrated in **Figure 10** using 30 equivalents of activated MPEG6 oligomers;

Figure 23 is an HPLC trace of a conjugation reaction of human growth hormone with the activated oligomer of **Figure 9**;

25 **Figure 24** is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and two equivalents of the activated oligomer of **Figure 9** according to the present invention compared with an HPLC trace of human growth hormone, which does not form part of the present invention;

30 **Figure 25** is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and five equivalents of the activated oligomer of **Figure 8** according to the present invention compared with an HPLC trace of human growth hormone, which does not form part of the present invention;

Figure 26 is a MALDI spectra of the fraction corresponding to the left half of the peak in the conjugation HPLC trace of **Figure 25**;

Figure 27 is a MALDI spectra of the fraction corresponding to the right half of the peak in the conjugation HPLC trace of **Figure 25**;

5 **Figure 28** is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and 9 equivalents of the activated oligomer of **Figure 8** according to the present invention compared with an HPLC trace of human growth hormone, which does not form part of the present invention;

Figure 29 illustrates a comparison of results obtained with a Cytosensor®

10 Microphysiometer, which provides an indication of the activity of a compound, for mixtures of insulin-oligomer conjugates according to embodiments of the present invention compared with polydispersed conjugate mixtures and insulin, which are provided for comparison purposes only and do not form part of the invention;

15 **Figure 30** illustrates a comparison of chymotrypsin degradation of insulin-oligomer conjugates according to embodiments of the present invention with a conventional polydispersed mixture of insulin-oligomer conjugates, which is provided for comparison purposes only and does not form part of the invention;

20 **Figure 31** illustrates the effect of a mixture of mPEG7-hexyl-insulin, monoconjugate, according to embodiments of the present invention on plasma glucose in fasted beagles;

25 **Figure 32** illustrates, for comparison purposes, the effect of a polydispersed mixture of mPEG7_{avg}-hexyl-insulin, monoconjugate, which is not part of the present invention, on plasma glucose in fasted beagles;

Figure 33 illustrates the inter-subject variability of a mixture of mPEG4-hexyl-insulin monoconjugates according to embodiments of the present invention administered to fasted beagles;

Figure 34 illustrates the inter-subject variability of a mixture of mPEG7-hexyl-insulin monoconjugates according to embodiments of the present invention administered to fasted beagles;

30 **Figure 35** illustrates the inter-subject variability for a mixture of mPEG10-hexyl-insulin monoconjugates according to embodiments of the present invention administered to fasted beagles;

Figure 36 illustrates, for comparison purposes, the inter-subject variability of a polydispersed mixture of mPEG7_{avg}-hexyl-insulin monoconjugates, which is not part of the present invention, administered to fasted beagles;

Figure 37 illustrates a comparison of the average AUCs for various monodispersed mixtures of calcitonin-oligomer conjugates according to embodiments of the present invention with non-conjugated calcitonin, which is provided for comparison purposes only and does not form part of the invention;

Figure 38 illustrates dose-response curves where the response is measured as a percentage of the maximum possible response for a mixture of mPEG7-octyl-calcitonin diconjugates according to embodiments of the present invention compared with calcitonin, which is provided for comparison purposes and is not a part of the present invention;

Figure 39 illustrates a dose-response curve after oral administration of a mixture of mPEG7-octyl-calcitonin diconjugates according to embodiments of the present invention;

Figure 40 illustrates a dose-response curve after subcutaneous administration of a mixture of mPEG7-octyl-calcitonin diconjugates according to embodiments of the present invention;

Figure 41 illustrates a dose-response curve after subcutaneous administration of salmon calcitonin, which is provided for comparison purposes and is not part of the present invention;

Figure 42 illustrates a bar graph denoting the activity as determined by luciferase assay of mixtures of growth hormone conjugates according to embodiments of the present invention compared with the activity of human growth hormone standards, which are provided for comparison purposes only and do not form part of the present invention; and

Figure 43 illustrates a bar graph denoting the activity as determined by luciferase assay of mixtures of growth hormone conjugates according to embodiments of the present invention compared with the activity of human growth hormone standards, which are provided for comparison purposes only and do not form part of the present invention.

Detailed Description Of Preferred Embodiments

The invention will now be described with respect to preferred embodiments described herein. It should be appreciated however that these embodiments are for the purpose of

illustrating the invention, and are not to be construed as limiting the scope of the invention as defined by the claims.

As used herein, the term "non-polydispersed" is used to describe a mixture of compounds having a dispersity that is in contrast to the polydispersed mixtures described in 5 U.S. Patent No. 4,179,337 to Davis et al.; U.S. Patent No. 5,567,422 to Greenwald; U.S. Patent No. 5,405,877 to Greenwald et al.; and U.S. Patent No. 5,359,030 to Ekwuribe.

As used herein, the term "substantially monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight.

10 As used herein, the term "monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "substantially purely monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight and same molecular structure. Thus, a substantially purely monodispersed mixture is a substantially monodispersed mixture, but a substantially monodispersed mixture is not necessarily a substantially purely monodispersed mixture. 15

As used herein, the term "purely monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight and have the same molecular structure. Thus, a purely monodispersed mixture is a monodispersed mixture, but a monodispersed mixture is not necessarily a purely monodispersed mixture. 20

As used herein, the term "weight average molecular weight" is defined as the sum of the products of the weight fraction for a given molecule in the mixture times the mass of the molecule for each molecule in the mixture. The "weight average molecular weight" is 25 represented by the symbol M_w .

As used herein, the term "number average molecular weight" is defined as the total weight of a mixture divided by the number of molecules in the mixture and is represented by the symbol M_n .

As used herein, the term "dispersity coefficient" (DC) is defined by the formula:

$$DC = \frac{\left(\sum_{i=1}^n N_i M_i \right)^2}{\sum_{i=1}^n N_i M_i^2 - \sum_{i=1}^n N_i \left(\sum_{i=1}^n N_i M_i \right)^2}$$

wherein:

n is the number of different molecules in the sample;

N_i is the number of ith molecules in the sample; and

M_i is the mass of the ith molecule.

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As used herein, the term "intra-subject variability" means the variability in activity occurring within the same subject when the subject is administered the same dose of a drug or pharmaceutical composition at different times.

As used herein, the term "inter-subject variability" means the variability in activity between two or more subjects when each subject is administered the same dose of a given drug or pharmaceutical formulation.

As used herein, the term "polyalkylene glycol" refers to straight or branched polyalkylene glycol polymers such as polyethylene glycol, polypropylene glycol, and polybutylene glycol, and includes the monoalkylether of the polyalkylene glycol. The term "polyalkylene glycol subunit" refers to a single polyalkylene glycol unit. For example, a polyethylene glycol subunit would be -O-CH₂-CH₂-O-.

As used herein, the term "lipophilic" means the ability to dissolve in lipids and/or the ability to penetrate, interact with and/or traverse biological membranes, and the term, "lipophilic moiety" or "lipophile" means a moiety which is lipophilic and/or which, when attached to another chemical entity, increases the lipophilicity of such chemical entity.

20 Examples of lipophilic moieties include, but are not limited to, alkyls, fatty acids, esters of fatty acids, cholestryl, adamantyl and the like.

As used herein, the term "lower alkyl" refers to substituted or unsubstituted alkyl moieties having from 1 to 5 carbon atoms.

25 As used herein, the term "higher alkyl" refers to substituted or unsubstituted alkyl moieties having 6 or more carbon atoms.

As used herein, the term "drug" refers to any therapeutic compound that is conjugatable in the manner of the present invention. Representative non-limiting classes of therapeutic compounds useful in the present invention include those falling into the following

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therapeutic categories: ACE-inhibitors; anti-anginal drugs; anti-arrhythmias; anti-asthmatics; anti-cholesterolemics; anti-convulsants; anti-depressants; anti-diarrhea preparations; anti-histamines; anti-hypertensive drugs; anti-infectives; anti-inflammatory agents; anti-lipid agents; anti-manics; anti-nauseants; anti-stroke agents; anti-thyroid preparations; anti-tumor drugs; anti-tussives; anti-uricemic drugs; anti-viral agents; acne drugs; alkaloids; amino acid preparations; anabolic drugs; analgesics; anesthetics; angiogenesis inhibitors; antacids; anti-arthritics; antibiotics; anticoagulants; antiemetics; antiobesity drugs; antiparasitics; antipsychotics; antipyretics; antispasmodics; antithrombotic drugs; anxiolytic agents; appetite stimulants; appetite suppressants; beta blocking agents; bronchodilators; cardiovascular agents; cerebral dilators; chelating agents; cholecystokinin antagonists; chemotherapeutic agents; cognition activators; contraceptives; coronary dilators; cough suppressants; decongestants; deodorants; dermatological agents; diabetes agents; diuretics; emollients; enzymes; erythropoietic drugs; expectorants; fertility agents; fungicides; gastrointestinal agents; growth regulators; hormone replacement agents; hyperglycemic agents; hypnotics; hypoglycemic agents; laxatives; migraine treatments; mineral supplements; mucolytics; narcotics; neuroleptics; neuromuscular drugs; NSAIDS; nutritional additives; peripheral vasodilators; polypeptides; prostaglandins; psychotropics; renin inhibitors; respiratory stimulants; steroids; stimulants; sympatholytics; thyroid preparations; tranquilizers; uterine relaxants; vaginal preparations; vasoconstrictors; vasodilators; vertigo agents; vitamins; and wound healing agents.

The drug is preferably a polypeptide. Non-limiting examples of polypeptides that may be useful in the present invention include the following:

Adrenocorticotrophic hormone (ACTH) peptides including, but not limited to, ACTH, human; ACTH 1-10; ACTH 1-13, human; ACTH 1-16, human; ACTH 1-17; ACTH 1-24, human; ACTH 4-10; ACTH 4-11; ACTH 6-24; ACTH 7-38, human; ACTH 18-39, human; ACTH, rat; ACTH 12-39, rat; beta-cell tropin (ACTH 22-39); biotinyl-ACTH 1-24, human; biotinyl-ACTH 7-38, human; corticostatin, human; corticostatin, rabbit; [Met(0)²]⁴, DLys⁸, Phe⁹] ACTH 4-9, human; [Met(0)⁴,DLys⁸, Phe⁹] ACTH 4-9, human; N-acetyl, ACTH 1-17, human; and ebiratide.

Adrenomedullin peptides including, but not limited to, adrenomedullin, adrenomedullin 1-52, human; adrenomedullin 1-12, human; adrenomedullin 13-52, human; adrenomedullin 22-52, human; pro-adrenomedullin 45-92, human; pro-adrenomedullin 153-

185, human; adrenomedullin 1-52, porcine; pro-adrenomedullin (N-20), porcine; adrenomedullin 1-50, rat; adrenomedullin 11-50, rat; and proAM-N20 (proadrenomedullin N-terminal 20 peptide), rat.

5 Allatostatin peptides including, but not limited to, allatostatin I; allatostatin II; allatostatin III; and allatostatin IV.

Amylin peptides including, but not limited to, acetyl-amylin 8-37, human; acetylated amylin 8-37, rat; AC187 amylin antagonist; AC253 amylin antagonist; AC625 amylin antagonist; amylin 8-37, human; amylin (IAPP), cat; amylin (insulinoma or islet amyloid polypeptide(IAPP)); amylin amide, human; amylin 1-13 (diabetes-associated peptide 1-13), 10 human; amylin 20-29 (IAPP 20-29), human; AC625 amylin antagonist; amylin 8-37, human; amylin (IAPP), cat; amylin, rat; amylin 8-37, rat; biotinyl-amylin, rat; and biotinyl-amylin amide, human.

Amyloid beta-protein fragment peptides including, but not limited to, Alzheimer's disease beta-protein 12-28 (SP17); amyloid beta-protein 25-35; amyloid beta/A4-protein precursor 328-332; amyloid beta/A4 protein precursor (APP) 319-335; amyloid beta-protein 1-43; amyloid beta-protein 1-42; amyloid beta-protein 1-40; amyloid beta-protein 10-20; amyloid beta-protein 22-35; Alzheimer's disease beta-protein (SP28); beta-amyloid peptide 1-42, rat; beta-amyloid peptide 1-40, rat; beta-amyloid 1-11; beta-amyloid 31-35; beta-amyloid 32-35; beta-amyloid 35-25; beta-amyloid/A4 protein precursor 96-110; beta-amyloid precursor protein 657-676; beta-amyloid 1-38; [Gln^{11}]-Alzheimer's disease beta-protein; [Gln^{11}]-beta-amyloid 1-40; [Gln^{22}]-beta-amyloid 6-40; non-A beta component of Alzheimer's disease amyloid (NAC); P3, (A beta 17-40) Alzheimer's disease amyloid β -peptide; and SAP (serum amyloid P component) 194-204.

Angiotensin peptides including, but not limited to, A-779; Ala-Pro-Gly-angiotensin 25 II; [$\text{Ile}^3, \text{Val}^5$]-angiotensin II; angiotensin III antipeptide; angiogenin fragment 108-122; angiogenin fragment 108-123; angiotensin I converting enzyme inhibitor; angiotensin I, human; angiotensin I converting enzyme substrate; angiotensin 11-7, human; angiopeptin; angiotensin II, human; angiotensin II antipeptide; angiotensin II 1-4, human; angiotensin II 3-8, human; angiotensin II 4-8, human; angiotensin II 5-8, human; angiotensin III ([Des-Asp¹]-angiotensin II), human; angiotensin III inhibitor ([Ile^7]-angiotensin III); angiotensin-converting enzyme inhibitor (Neothunnus macropterus); [$\text{Asn}^1, \text{Val}^5$]-angiotensin I, goosefish; [$\text{Asn}^1, \text{Val}^5, \text{Asn}^9$]-angiotensin I, salmon; [$\text{Asn}^1, \text{Val}^5, \text{Gly}^9$]-angiotensin I, eel;

[Asn¹, Val⁵]-angiotensin I 1-7, eel, goosefish, salmon; [Asn¹, Val⁵]-angiotensin II; biotinyl-angiotensin I, human; biotinyl-angiotensin II, human; biotinyl-Ala-Ala-Ala-angiotensin II; [Des-Asp¹]-angiotensin I, human; [p-aminophenylalanine⁶]-angiotensin II; renin substrate (angiotensinogen 1-13), human; preangiotensinogen 1-14 (renin substrate tetradecapeptide),
5 human; renin substrate tetradecapeptide (angiotensinogen 1-14), porcine; [Sar¹]-angiotensin II, [Sar¹]-angiotensin II 1-7 amide; [Sar¹, Ala⁸]-angiotensin II; [Sar¹, Ile⁸]-angiotensin II; [Sar¹, Thr⁸]-angiotensin II; [Sar¹, Tyr(Me)⁴]-angiotensin II (Sarmesin); [Sar¹, Val⁵, Ala⁸]-angiotensin II; [Sar¹, Ile⁷]-angiotensin III; synthetic tetradecapeptide renin substrate (No. 2); [Val⁴]-angiotensin III; [Val⁵]-angiotensin II; [Val⁵]-angiotensin I, human; [Val⁵]-angiotensin I; [Val⁵, Asn⁹]-angiotensin I, bullfrog; and [Val⁵, Ser⁹]-angiotensin I, fowl.

10 Antibiotic peptides including, but not limited to, Ac-SQNY; bactenecin, bovine; CAP 37 (20-44); carbormethoxycarbonyl-DPro-DPhe-OBzl; CD36 peptide P 139-155; CD36 peptide P 93-110; cecropin A-melittin hybrid peptide [CA(1-7)M(2-9)NH₂]; cecropin B, free acid; CYS(Bzl)84 CD fragment 81-92; defensin (human) HNP-2; dermaseptin; immunostimulating peptide, human; lactoferricin, bovine (BLFC); and magainin spacer.

15 Antigenic polypeptides, which can elicit an enhanced immune response, enhance an immune response and or cause an immunizingly effective response to diseases and/or disease causing agents including, but not limited to, adenoviruses; anthrax; Bordetella pertussus; botulism; bovine rhinotracheitis; Branhamella catarrhalis; canine hepatitis; canine distemper; Chlamydiae; cholera; coccidiomycosis; cowpox; cytomegalovirus; Dengue fever; dengue toxoplasmosis; diphtheria; encephalitis; enterotoxigenic E. coli; Epstein Barr virus; equine encephalitis; equine infectious anemia; equine influenza; equine pneumonia; equine rhinovirus; Escherichia coli; feline leukemia; flavivirus; globulin; haemophilus influenza type b; Haemophilus influenzae; Haemophilus pertussis; Helicobacter pylon; hemophilus;
20 hepatitis; hepatitis A; hepatitis B; Hepatitis C; herpes viruses; HIV; HIV- 1 viruses; HIV-2 viruses; HTLV; influenza; Japanese encephalitis; Klebsiellae species; Legionella pneumophila; leishmania; leprosy; lyme disease; malaria immunogen; measles; meningitis; meningococcal; Meningococcal polysaccharide group A; Meningococcal polysaccharide group C; mumps; mumps virus; mycobacteria; Mycobacterium tuberculosis; Neisseria;
25 Neisseria gonorrhoea; Neisseria meningitidis; ovine blue tongue; ovine encephalitis; papilloma; parainfluenza; paramyxoviruses; Pertussis; plague; pneumococcus; Pneumocystis carinii; pneumonia; poliovirus; proteus species; Pseudomonas aeruginosa; rabies; respiratory

syncytial virus; rotavirus; rubella; salmonellae; schistosomiasis; shigellae; simian immunodeficiency virus; smallpox; *Staphylococcus aureus*; *Staphylococcus* species; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Streptococcus* species; swine influenza; tetanus; *Treponema pallidum*; typhoid; vaccinia; varicella-zoster virus; and vibrio cholerae.

5 Anti-microbial peptides including, but not limited to, buforin I; buforin II; cecropin A; cecropin B; cecropin P1, porcine; gaegurin 2 (*Rana rugosa*); gaegurin 5 (*Rana rugosa*); indolicidin; protegrin-(PG)-I; magainin 1; and magainin 2; and T-22 [$\text{Tyr}^{5,12}$, Lys^7]-polyphemusin II peptide.

10 Apoptosis related peptides including, but not limited to, Alzheimer's disease beta-protein (SP28); calpain inhibitor peptide; caspase-1 inhibitor V; caspase-3, substrate IV; caspase-1 inhibitor I, cell-permeable; caspase-1 inhibitor VI; caspase-3 substrate III, fluorogenic; caspase-1 substrate V, fluorogenic; caspase-3 inhibitor I, cell-permeable; caspase-6 ICE inhibitor III; [Des-Ac, biotin]-ICE inhibitor III; IL-1 B converting enzyme (ICE) inhibitor II; IL-1 B converting enzyme (ICE) substrate IV; MDL 28170; and MG-132.

15 Atrial natriuretic peptides including, but not limited to, alpha-ANP (alpha-chANP), chicken; anantin; ANP 1-11, rat; ANP 8-30, frog; ANP 11-30, frog; ANP-21 (fANP-21), frog; ANP-24 (fANP-24), frog; ANP-30, frog; ANP fragment 5-28, human, canine; ANP-7-23, human; ANP fragment 7-28, human, canine; alpha-atrial natriuretic polypeptide 1-28, human, canine; A71915, rat; atrial natriuretic factor 8-33, rat; atrial natriuretic polypeptide 3-28, human; atrial natriuretic polypeptide 4-28, human, canine; atrial natriuretic polypeptide 5-27; human; atrial natriuretic aep tide (ANP), eel; atriopeptin I, rat, rabbit, mouse; atriopeptin II, rat, rabbit, mouse; atriopeptin III, rat, rabbit, mouse; atrial natriuretic factor (rANF), rat, auriculin A (rat ANF 126-149); auriculin B (rat ANF 126-150); beta-ANP (1-28, dimer, antiparallel); beta-rANF 17-48; biotinyl-alpha-ANP 1-28, human, canine; biotinyl-atrial 20 natriuretic factor (biotinyl-rANF), rat; cardiodilatin 1-16, human; C-ANF 4-23, rat; Des-[Cys¹⁰⁵, Cys¹²¹]-atrial natriuretic factor 104-126, rat; [Met(O)¹²] ANP 1-28, human; [Mpr⁷,DAla⁹]ANP 7-28, amide, rat; prepro-ANF 104-116, human; prepro-ANF 26-55 (proANF 1-30), human; prepro-ANF 56-92 (proANF 31-67), human; prepro-ANF 104-123, human; [Tyr^0]-atriopeptin I, rat, rabbit, mouse; [Tyr^0]-atriopeptin II, rat, rabbit, mouse; 25 [Tyr⁰]-prepro ANF 104-123, human; urodilatin (CDD/ANP 95-126); ventricular natriuretic peptide (VNP), eel; and ventricular natriuretic peptide (VNP), rainbow trout.

Bag cell peptides including, but not limited to, alpha bag cell peptide; alpha-bag cell peptide 1-9; alpha-bag cell peptide 1-8; alpha-bag cell peptide 1-7; beta-bag cell factor; and gamma-bag cell factor.

Bombesin peptides including, but not limited to, alpha-s1 casein 101-123 (bovine milk); biotinyl-bombesin; bombesin 8-14; bombesin; [Leu¹³-psi (CH₂NH)Leu¹⁴]-bombesin; [D-Phe⁶, Des-Met¹⁴]-bombesin 6-14 ethylamide; [DPhe¹²] bombesin; [DPhe¹²,Leu¹⁴]-bombesin; [Tyr⁴]-bombesin; and [Tyr⁴,DPhe¹²]-bombesin.

Bone GLA peptides (BGP) including, but not limited to, bone GLA protein; bone GLA protein 45-49; [Glu¹⁷, Gla^{21,24}]-osteocalcin 1-49, human; myclopeptide -2 (MP-2); osteocalcin 1-49 human; osteocalcin 37-49, human; and [Tyr³⁸, Phe^{42,46}] bone GLA protein 38-49, human.

Bradykinin peptides including, but not limited to, [Ala^{2,6}, des-Pro³]-bradykinin; bradykinin; bradykinin (Bowfin. Gar); bradykinin potentiating peptide; bradykinin 1-3; bradykinin 1-5; bradykinin 1-6; bradykinin 1-7; bradykinin 2-7; bradykinin 2-9; [DPhe⁷] bradykinin; [Des-Arg⁹]-bradykinin; [Des-Arg¹⁰]-Lys-bradykinin ([Des-Arg¹⁰]-kallidin); [D-N-Me-Phe⁷]-bradykinin; [Des-Arg⁹, Leu⁸]-bradykinin; Lys-bradykinin (kallidin); Lys-[Des-Arg⁹,Leu⁸]-bradykinin ([Des-Arg¹⁰,Leu⁹]-kallidin); [Lys⁰-Hyp³]-bradykinin; ovokinin; [Lys⁰, Ala³]-bradykinin; Met-Lys-bradykinin; peptide K12 bradykinin potentiating peptide; [(pCl)Phe^{5,8}]-bradykinin; T-kinin (Ile-Ser-bradykinin); [Thi^{5,8}, D-Phe⁷]-bradykinin; [Tyr⁰]-bradykinin; [Tyr⁵]-bradykinin; [Tyr⁸]-bradykinin; and kallikrein.

Brain natriuretic peptides (BNP) including, but not limited to, BNP 32, canine; BNP-like Peptide, eel; BNP-32, human; BNP-45, mouse; BNP-26, porcine; BNP-32, porcine; biotinyl-BNP-32, porcine; BNP-32, rat; biotinyl-BNP-32, rat; BNP-45 (BNP 51-95, 5K cardiac natriuretic peptide), rat; and [Tyr⁰]-BNP 1-32, human.

C-peptides including, but not limited to, C-peptide; and [Tyr⁰]-C-peptide, human.

C-type natriuretic peptides (CNP) including, but not limited to, C-type natriuretic peptide, chicken; C-type natriuretic peptide-22 (CNP-22), porcine, rat, human; C-type natriuretic peptide-53 (CNP-53), human; C-type natriuretic peptide-53 (CNP-53), porcine, rat; C-type natriuretic peptide-53 (porcine, rat) 1-29 (CNP-53 1-29); prepro-CNP 1-27, rat; prepro-CNP 30-50, porcine, rat; vasonatrin peptide (VNP); and [Tyr⁰]-C-type natriuretic peptide-22 ([Tyr⁰]-CNP-22).

Calcitonin peptides including, but not limited to, biotinyl-calcitonin, human; biotinyl-calcitonin, rat; biotinyl-calcitonin, salmon; calcitonin, chicken; calcitonin, eel; calcitonin, human; calcitonin, porcine; calcitonin, rat; calcitonin, salmon; calcitonin 1-7, human; calcitonin 8-32, salmon; katacalcin (PDN-21) (C-procalcitonin); and N-proCT (amino-terminal procalcitonin cleavage peptide), human.

Calcitonin gene related peptides (CGRP) including, but not limited to, acetyl-alpha-CGRP 19-37, human; alpha-CGRP 19-37, human; alpha-CGRP 23-37, human; biotinyl-CGRP, human; biotinyl-CGRP II, human; biotinyl-CGRP, rat; beta-CGRP, rat; biotinyl-beta-CGRP, rat; CGRP, rat; CGRP, human; calcitonin C-terminal adjacent peptide; CGRP 1-19, human; CGRP 20-37, human; CGRP 8-37, human; CGRP II, human; CGRP, rat; CGRP 8-37, rat; CGRP 29-37, rat; CGRP 30-37, rat; CGRP 31-37, rat; CGRP 32-37, rat; CGRP 33-37, rat; CGRP 31-37, rat; ($[Cys(Acm)^{2,7}]$ -CGRP; elcatonin; $[Tyr^0]$ -CGRP, human; $[Tyr^0]$ -CGRP II, human; $[Tyr^0]$ -CGRP 28-37, rat; $[Tyr^0]$ -CGRP, rat; and $[Tyr^{22}]$ -CGRP 22-37, rat.

CART peptides including, but not limited to, CART, human; CART 55-102, human; CART, rat; and CART 55-102, rat.

Casomorphin peptides including, but not limited to, beta-casomorphin, human; beta-casomorphin 1-3; beta-casomorphin 1-3, amide; beta-casomorphin, bovine; beta-casomorphin 1-4, bovine; beta-casomorphin 1-5, bovine; beta-casomorphin 1-5, amide, bovine; beta-casomorphin 1-6, bovine; $[DAla^2]$ -beta-casomorphin 1-3, amide, bovine; $[DAla^2, Hyp^4, Tyr^5]$ -beta-casomorphin 1-5 amide; $[DAla^2, DPro^4, Tyr^5]$ -beta-casomorphin 1-5, amide; $[DAla^2, Tyr^5]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^{2,4}, Tyr^5]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2, (pCl)Phe^3]$ -beta-casomorphin, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-4, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-5, bovine; $[DAla^2]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2, Met^5]$ -beta-casomorphin 1-5, bovine; $[DPro^2]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-6, bovine; $[DPro^2]$ -beta-casomorphin 1-4, amide; $[Des-Tyr^1]$ -beta-casomorphin, bovine; $[DAla^{2,4}, Tyr^5]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2, (pCl)Phe^3]$ -beta-casomorphin, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-4, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-5, bovine; $[DAla^2]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2, Met^5]$ -beta-casomorphin 1-5, bovine; $[DPro^2]$ -beta-casomorphin 1-5, amide, bovine; $[DAla^2]$ -beta-casomorphin 1-6, bovine; $[DPro^2]$ -beta-casomorphin 1-4, amide; $[Des-Tyr^1]$ -beta-casomorphin, bovine; and $[Val^3]$ -beta-casomorphin 1-4, amide, bovine.

Chemotactic peptides including, but not limited to, defensin 1 (human) HNP-1 (human neutrophil peptide-1); and N-formyl-Met-Leu-Phe.

Cholecystokinin (CCK) peptides including, but not limited to, caerulein; cholecystokinin; cholecystokinin-pancreozymin; CCK-33, human; cholecystokinin 5 octapeptide 1-4 (non-sulfated) (CCK 26-29, unsulfated); cholecystokinin octapeptide (CCK 26-33); cholecystokinin octapeptide (non-sulfated) (CCK 26-33, unsulfated); cholecystokinin heptapeptide (CCK 27-33); cholecystokinin tetrapeptide (CCK 30-33); CCK-33, porcine; CR 1 409, cholecystokinin antagonist; CCK flanking peptide (unsulfated); N-acetyl cholecystokinin, CCK 26-30, sulfated; N-acetyl cholecystokinin, CCK 26-31, sulfated; N-10 acetyl cholecystokinin, CCK 26-31, non-sulfated; prepro CCK fragment V-9-M; and proglumide.

Colony-stimulating factor peptides including, but not limited to, colony-stimulating factor (CSF); GMCSF; MCSF; and G-CSF.

Corticotropin releasing factor (CRF) peptides including, but not limited to, astressin; alpha-helical CRF 12-41; biotinyl-CRF, ovine; biotinyl-CRF, human, rat; CRF, bovine; CRF, human, rat; CRF, ovine; CRF, porcine; [Cys²¹]-CRF, human, rat; CRF antagonist (alpha-helical CRF 9-41); CRF 6-33, human, rat; [DPro⁵]-CRF, human, rat; [D-Phe¹², Nle^{21,38}]-CRF 12-41, human, rat; eosinophilotactic peptide; [Met(0)²¹]-CRF, ovine; [Nle²¹,Tyr³²]-CRF, ovine; prepro CRF 125-151, human; sauvagine, frog; [Tyr⁰]-CRF, human, rat; [Tyr⁰]-CRF, ovine; [Tyr⁰]-CRF 34-41, ovine; [Tyr⁰]-urocortin; urocortin amide, human; urocortin, rat; urotensin I (*Catostomus commersoni*); urotensin II; and urotensin II (*Rana ridibunda*).

Cortistatin peptides including, but not limited to, cortistatin 29; cortistatin 29 (1-13); [Tyr⁰]-cortistatin 29; pro-cortistatin 28-47; and pro-cortistatin 51-81.

Cytokine peptides including, but not limited to, tumor necrosis factor; and tumor 25 necrosis factor- β (TNF- β).

Dermorphin peptides including, but not limited to, dermorphin and dermorphin analog 1-4.

Dynorphin peptides including, but not limited to, big dynorphin (prodynorphin 209-240), porcine; biotinyl-dynorphin A (biotinyl-prodynorphin 209-225); [DAla², DArg⁶]-30 dynorphin A 1-13, porcine; [D-Ala²]-dynorphin A, porcine; [D-Ala²]-dynorphin A amide, porcine; [D-Ala²]-dynorphin A 1-13, amide, porcine; [D-Ala²]-dynorphin A 1-9, porcine; [DArg⁶]-dynorphin A 1-13, porcine; [DArg⁸]-dynorphin A 1-13, porcine; [Des-Tyr¹]-

dynorphin A 1-8; [D-Pro¹⁰]-dynorphin A 1-11, porcine; dynorphin A amide, porcine;
dynorphin A 1-6, porcine; dynorphin A 1-7, porcine; dynorphin A 1-8, porcine; dynorphin A
1-9, porcine; dynorphin A 1-10, porcine; dynorphin A 1-10 amide, porcine; dynorphin A 1-
11, porcine; dynorphin A 1-12, porcine; dynorphin A 1-13, porcine; dynorphin A 1-13 amide,
5 porcine; DAKLI (dynorphin A-analogue kappa ligand); DAKLI-biotin ([Arg^{11,13}]-dynorphin
A (1-13)-Gly-NH(CH₂)₅NH-biotin); dynorphin A 2-17, porcine; dynorphin 2-17, amide,
porcine; dynorphin A 2-12, porcine; dynorphin A 3-17, amide, porcine; dynorphin A 3-8,
porcine; dynorphin A 3-13, porcine; dynorphin A 3-17, porcine; dynorphin A 7-17, porcine;
dynorphin A 8-17, porcine; dynorphin A 6-17, porcine; dynorphin A 13-17, porcine;
10 dynorphin A (prodynorphin 209-225), porcine; dynorphin B 1-9; [MeTyr¹, MeArg⁷, D-Leu⁸]-
dynorphin 1-8 ethyl amide; [(nMe)Tyr¹] dynorphin A 1-13, amide, porcine; [Phe⁷]-dynorphin
A 1-7, porcine; [Phe⁷]-dynorphin A 1-7, amide, porcine; and prodynorphin 228-256
(dynorphin B 29) (leumorphin), porcine.

Endorphin peptides including, but not limited to, alpha-neo-endorphin, porcine; beta-
15 neo-endorphin; Ac-beta-endorphin, camel, bovine, ovine; Ac-beta-endorphin 1-27, camel,
bovine, ovine; Ac-beta-endorphin, human; Ac-beta-endorphin 1-26, human; Ac-beta-
endorphin 1-27, human; Ac-gamma-endorphin (Ac-beta-lipotropin 61-77); acetyl-alpha-
endorphin; alpha-endorphin (beta-lipotropin 61-76); alpha-neo-endorphin analog; alpha-neo-
endorphin 1-7; [Arg⁸]-alpha-neo-endorphin 1-8; beta-endorphin (beta-lipotropin 61-91),
20 camel, bovine, ovine; beta-endorphin 1-27, camel, bovine, ovine; beta-endorphin, equine;
beta-endorphin (beta-lipotropin 61-91), human; beta-endorphin (1-5) + (16-31), human; beta-
endorphin 1-26, human; beta-endorphin 1-27, human; beta-endorphin 6-31, human; beta-
endorphin 18-31, human; beta-endorphin, porcine; beta-endorphin, rat; beta-lipotropin 1-10,
25 porcine; beta-lipotropin 60-65; beta-lipotropin 61-64; beta-lipotropin 61-69; beta-lipotropin
88-91; biotinyl-beta-endorphin (biotinyl-beta-lipotropin 61-91); biocytin-beta-endorphin,
human; gamma-endorphin (beta-lipotropin 61-77); [DAla²]-alpha-neo-endorphin 1-2, amide;
[DAla²]-beta-lipotropin 61-69; [DAla²]-gamma-endorphin; [Des-Tyr¹]-beta-endorphin,
30 human; [Des-Tyr¹]-gamma-endorphin (beta-lipotropin 62-77); [Leu⁵]-beta-endorphin, camel,
bovine, ovine; [Met⁵, Lys⁶]-alpha-neo-endorphin 1-6; [Met⁵, Lys^{6,7}]-alpha-neo-endorphin 1-
7; and [Met⁵, Lys⁶, Arg⁷]-alpha-neo-endorphin 1-7.

Endothelin peptides including, but not limited to, endothelin-1 (ET-1); endothelin-
1[Biotin-Lys⁹]; endothelin-1 (1-15), human; endothelin-1 (1-15), amide, human; Ac-

endothelin-1 (16-21), human; Ac-[DTrp¹⁶]-endothelin-1 (16-21), human; [Ala^{3,11}]-endothelin-1; [Dprl, Asp¹⁵]-endothelin-1; [Ala²]-endothelin-3, human; [Ala¹⁸]-endothelin-1, human; [Asn¹⁸]-endothelin-1, human; [Res-701-1]-endothelin B receptor antagonist; Suc-[Glu⁹, Ala^{11,15}]-endothelin-1 (8-21), IRL-1620; endothelin-C-terminal hexapeptide; [D-Val²²]-big endothelin-1 (16-38), human; endothelin-2 (ET-2), human, canine; endothelin-3 (ET-3), human, rat, porcine, rabbit; biotinyl-endothelin-3 (biotinyl-ET-3); prepro-endothelin-1 (94-109), porcine; BQ-518; BQ-610; BQ-788; endothelium-dependent relaxation antagonist; FR139317; IRL-1038; JKC-30 1; JKC-302; PD-145065; PD 142893; sarafotoxin S6a (atractaspis engaddensis); sarafotoxin S6b (atractaspis engaddensis); sarafotoxin S6c (atractaspis engaddensis); [Lys⁴]-sarafotoxin S6c; sarafotoxin S6d; big endothelin-1, human; biotinyl-big endothelin-1, human; big endothelin-1 (1-39), porcine; big endothelin-3 (22-41), amide, human; big endothelin-1 (22-39), rat; big endothelin-1 (1-39), bovine; big endothelin-1 (22-39), bovine; big endothelin-1 (19-38), human; big endothelin-1 (22-38), human; big endothelin-2, human; big endothelin-2 (22-37), human; big endothelin-3, human; big endothelin-1, porcine; big endothelin-1 (22-39) (prepro-endothelin-1 (74-91)); big endothelin-1, rat; big endothelin-2 (1-38), human; big endothelin-2 (22-38), human; big endothelin-3, rat; biotinyl-big endothelin-1, human; and [Tyr¹²³]-prepro-endothelin (110-130), amide, human.

ETa receptor antagonist peptides including, but not limited to, [BQ-123];

[BE18257B]; [BE-18257A]/[W-7338A]; [BQ-485]; FR139317; PD-151242; and TTA-386.

ETb receptor antagonist peptides including, but not limited to, [BQ-3020]; [RES-701-3]; and [IRL-1720]

Enkephalin peptides including, but not limited to, adrenorphin, free acid; amidorphin (proenkephalin A (104-129)-NH2), bovine; BAM-12P (bovine adrenal medulla dodecapeptide); BAM-22P (bovine adrenal medulla docosapeptide); benzoyl-Phe- Ala-Arg; enkephalin; [D-Ala², D-Leu⁵]-enkephalin; [D-Ala², D-Met⁵]-enkephalin; [DAla²]-Leu-enkephalin, amide; [DAla²,Leu⁵,Arg⁶]-enkephalin; [Des-Tyr¹,DPen^{2,5}]-enkephalin; [Des-Tyr¹,DPen²,Pen⁵]-enkephalin; [Des-Tyr¹]-Leu-enkephalin; [D-Pen^{2,5}]-enkephalin; [DPen², Pen⁵]-enkephalin; enkephalinase substrate; [D-Pen², pCI-Phe⁴, D-Pen⁵]-enkephalin; Leu-enkephalin; Leu-enkephalin, amide; biotinyl-Leu-enkephalin; [D-Ala²]-Leu-enkephalin; [D-Ser²]-Leu-enkephalin-Thr (delta-receptor peptide) (DSLET); [D-Thr²]-Leu-enkephalin-Thr (DTLET); [Lys⁶]-Leu-enkephalin; [Met⁵,Arg⁶]-enkephalin; [Met⁵,Arg⁶]-enkephalin-Arg;

[Met⁵,Arg⁶,Phe⁷]-enkephalin, amide; Met-enkephalin; biotinyl-Met-enkephalin; [D-Ala²]-Met-enkephalin; [D-Ala²]-Met-enkephalin, amide; Met-enkephalin-Arg-Phe; Met-enkephalin, amide; [Ala²]-Met-enkephalin, amide; [DMet²,Pro⁵]-enkephalin, amide; [DTrp²]-Met-enkephalin, amide, metorphinamide (adrenorphin); peptide B, bovine; 3200-Dalton
5 adrenal peptide E, bovine; peptide F, bovine; preproenkephalin B 186-204, human; spinorphin, bovine; and thiorphan (D, L, 3-mercaptopropanoyl-glycine).

Fibronectin peptides including, but not limited to platelet factor-4 (58-70), human; echistatin (Echis carinatus); E, P ,L selectin conserved region; fibronectin analog; fibronectin-binding protein; fibrinopeptide A, human; [Tyr⁰]-fibrinopeptide A, human;
10 fibrinopeptide B, human; [Glu¹]-fibrinopeptide B, human; [Tyr¹⁵]-fibrinopeptide B, human; fibrinogen beta-chain fragment of 24-42; fibrinogen binding inhibitor peptide; fibronectin related peptide (collagen binding fragment); fibrinolysis inhibiting factor; FN-C/H-1 (fibronectin heparin-binding fragment); FN-C/H-V (fibronectin heparin-binding fragment); heparin-binding peptide; laminin penta peptide, amide; Leu-Asp-Val-NH2 (LDV-NH2),
15 human, bovine, rat, chicken; necrofibrin, human; necrofibrin, rat; and platelet membrane glycoprotein IIB peptide 296-306.

Galanin peptides including, but not limited to, galanin, human; galanin 1-19, human; preprogalanin 1-30, human; preprogalanin 65-88, human; preprogalanin 89-123, human; galanin, porcine; galanin 1-16, porcine, rat; galanin, rat; biotinyl-galanin, rat; preprogalanin
20 28-67, rat; galanin 1-13-bradykinin 2-9, amide; M40, galanin 1-13-Pro-Pro-(Ala-Leu) 2-Ala amide; C7, galanin 1-13-spantide-amide; GMAP 1-41, amide; GMAP 16-41, amide; GMAP 25-41, amide; galantide; and entero-kassinin.

Gastrin peptides including, but not limited to, gastrin, chicken; gastric inhibitory peptide (GIP), human; gastrin I, human; biotinyl-gastrin I, human; big gastrin-1, human;
25 gastrin releasing peptide, human; gastrin releasing peptide 1-16, human; gastric inhibitory polypeptide (GIP), porcine; gastrin releasing peptide, porcine; biotinyl-gastrin releasing peptide, porcine; gastrin releasing peptide 14-27, porcine, human; little gastrin, rat; pentagastrin; gastric inhibitory peptide 1-30, porcine; gastric inhibitory peptide 1-30, amide, porcine; [Tyr⁰]-gastric inhibitory peptide 23-42, human; and gastric inhibitory peptide, rat.

30 Glucagon peptides including, but not limited to, [Des-His¹,Glu⁹]-glucagon, extendin-4, glucagon, human; biotinyl-glucagon, human; glucagon 19-29, human; glucagon 22-29, human; Des-His¹-[Glu⁹]-glucagon, amide; glucagon-like peptide 1, amide (preproglucagon

72-107, amide); glucagon-like peptide 1 (preproglucagon 72-108), human; glucagon-like peptide 1 (7-36) (preproglucagon 78-107, amide); glucagon-like peptide II, rat; biotinyl-glucagon-like peptide-1 (7-36) (biotinyl-preproglucagon 78-107, amide); glucagon-like peptide 2 (preproglucagon 126-159), human; oxyntomodulin/glucagon 37; and valosin

5 (peptide VQY), porcine.

Gn-RH associated peptides (GAP) including, but not limited to, Gn-RH associated peptide 25-53, human; Gn-RH associated peptide 1-24, human; Gn-RH associated peptide 1-13, human; Gn-RH associated peptide 1-13, rat; gonadotropin releasing peptide, follicular, human; [Tyr^0]-GAP ([Tyr^0]-Gn-RH Precursor Peptide 14-69), human; and

10 proopiomelanocortin (POMC) precursor 27-52, porcine.

Growth factor peptides including, but not limited to, cell growth factors; epidermal growth factors; tumor growth factor; alpha-TGF; beta-TF; alpha-TGF 34-43, rat; EGF, human; acidic fibroblast growth factor; basic fibroblast growth factor; basic fibroblast growth factor 13-18; basic fibroblast growth factor 120-125; brain derived acidic fibroblast growth factor 1-11; brain derived basic fibroblast growth factor 1-24; brain derived acidic fibroblast growth factor 102-111; [$Cys(Acm^{20,31})$]-epidermal growth factor 20-31; epidermal growth factor receptor peptide 985-996; insulin-like growth factor (IGF)-I, chicken; IGF-I, rat; IGF-I, human; Des (1-3) IGF-I, human; R3 IGF-I, human; R3 IGF-I, human; long R3 IGF-I, human; adjuvant peptide analog; anorexigenic peptide; Des (1-6) IGF-II, human; R6 IGF-II, human; IGF-I analogue; IGF I (24-41); IGF I (57-70); IGF I (30-41); IGF II; IGF II (33-40); [Tyr^0]-IGF II (33-40); liver cell growth factor; midkine; midkine 60-121, human; N-acetyl, alpha-TGF 34-43, methyl ester, rat; nerve growth factor (NGF), mouse; platelet-derived growth factor; platelet-derived growth factor antagonist; transforming growth factor-alpha, human; and transforming growth factor-I, rat.

25 Growth hormone peptides including, but not limited to, growth hormone (hGH), human; growth hormone 1-43, human; growth hormone 6-13, human; growth hormone releasing factor, human; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; growth hormone releasing factor 1-29, amide, rat; growth hormone pro-releasing factor, human; biotinyl-growth hormone releasing factor, human; growth hormone releasing factor 1-29, amide, human; [$D\text{-Ala}^2$]-growth hormone releasing factor 1-29, amide, human; [$N\text{-Ac-Tyr}^1, D\text{-Arg}^2$]-GRF 1-29, amide; [His^1, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing factor 1-37, human; growth hormone releasing

factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; biotinyl- growth hormone releasing factor, rat; GHRP-6 ([His¹, Lys⁶]-GHRP); hexarelin (growth hormone releasing hexapeptide); and [D-Lys³]-GHRP-6.

5 GTP-binding protein fragment peptides including, but not limited to, [Arg⁸]-GTP-binding protein fragment, Gs alpha; GTP-binding protein fragment, G beta; GTP-binding protein fragment, GAlpha; GTP-binding protein fragment, Go Alpha; GTP-binding protein fragment, Gs Alpha; and GTP-binding protein fragment, G Alpha i2.

10 Guanylin peptides including, but not limited to, guanylin, human; guanylin, rat; and uroguanylin.

15 Inhibin peptides including, but not limited to, inhibin, bovine; inhibin, alpha-subunit 1-32, human; [Tyr⁰]-inhibin, alpha-subunit 1-32, human; seminal plasma inhibin-like peptide, human; [Tyr⁰]-seminal plasma inhibin-like peptide, human; inhibin, alpha-subunit 1-32, porcine; and [Tyr⁰]-inhibin, alpha-subunit 1-32, porcine.

20 Insulin peptides including, but not limited to, insulin, human; insulin, porcine; IGF-I, human; insulin-like growth factor II (69-84); pro-insulin-like growth factor II (68-102), human; pro-insulin-like growth factor II (105-128), human; [Asp^{B28}]-insulin, human; [Lys^{B28}]-insulin, human; [Leu^{B28}]-insulin, human; [Val^{B28}]-insulin, human; [Ala^{B28}]-insulin, human; [Asp^{B28}, Pro^{B29}]-insulin, human; [Lys^{B28}, Pro^{B29}]-insulin, human; [Leu^{B28}, Pro^{B29}]-insulin, human; [Val^{B28}, Pro^{B29}]-insulin, human; and [Ala^{B28}, Pro^{B29}]-insulin, human, B22-B30 insulin, human; B23-B30 insulin, human; B25-B30 insulin, human; B26-B30 insulin, human; B27-B30 insulin, human; B29-B30 insulin, human; the A chain of human insulin, and the B chain of human insulin.

25 Interleukin peptides including, but not limited to, interleukin-1 beta 165-181, rat; and interleukin-8 (IL-8, CINC/gro), rat.

Laminin peptides including, but not limited to, laminin; alpha1 (I)-CB3 435-438, rat; and laminin binding inhibitor.

30 Leptin peptides including, but not limited to, leptin 93-105, human; leptin 22-56, rat; Tyr-leptin 26-39, human; and leptin 116-130, amide, mouse.

Leucokinin peptides including, but not limited to, leucomyosuppressin (LMS); leucopyrokinin (LPK); leucokinin I; leucokinin II; leucokinin III; leucokinin IV; leucokinin VI; leucokinin VII; and leucokinin VIII.

- Luteinizing hormone-releasing hormone peptides including, but not limited to, antide;
- 5 Gn-RH II, chicken; luteinizing hormone-releasing hormone (LH-RH) (GnRH); biotinyl-LH-RH; cetrorelix (D-20761); [D-Ala⁶]-LH-RH; [Gln⁸]-LH-RH (Chicken LH-RH); [DLeu⁶, Val⁷] LH-RH 1-9, ethyl amide; [D-Lys⁶]-LH-RH; [D-Phe², Pro³, D-Phe⁶]-LH-RH; [DPhe², DAla⁶] LH-RH; [Des-Gly¹⁰]-LH-RH, ethyl amide; [D-Ala⁶, Des-Gly¹⁰]-LH-RH, ethyl amide; [DTrp⁶]-LH-RH, ethyl amide; [D-Trp⁶, Des-Gly¹⁰]-LH-RH, ethyl amide (Deslorelin);
- 10 [DSer(But)⁶, Des-Gly¹⁰]-LH-RH, ethyl amide; ethyl amide; leuprolide; LH-RH 4-10; LH-RH 7-10; LH-RH, free acid; LH-RH, lamprey; LH-RH, salmon; [Lys⁸]-LH-RH; [Trp⁷,Leu⁸] LH-RH, free acid; and [(t-Bu)DSer⁶, (Aza)Gly¹⁰]-LH-RH.

Mastoparan peptides including, but not limited to, mastoparan; mas7; mas8; mas17; and mastoparan X.

15 Mast cell degranulating peptides including, but not limited to, mast cell degranulating peptide HR-1; and mast cell degranulating peptide HR-2.

Melanocyte stimulating hormone (MSH) peptides including, but not limited to, [Ac-Cys⁴,DPhen⁷,Cys¹⁰] alpha-MSH 4-13, amide; alpha-melanocyte stimulating hormone; alpha-MSH, free acid; beta-MSH, porcine; biotinyl-alpha-melanocyte stimulating hormone; biotinyl-[Nle⁴, D-Phe⁷] alpha-melanocyte stimulating hormone; [Des -Acetyl]-alpha-MSH; [DPhen⁷]-alpha-MSH, amide; gamma-1-MSH, amide; [Lys⁹]-gamma-1-MSH, amide; MSH release inhibiting factor, amide; [Nle⁴]-alpha-MSH, amide; [Nle⁴, D-Phe⁷]-alpha-MSH; N-Acetyl, [Nle⁴,DPhen⁷] alpha-MSH 4-10, amide; beta-MSH, human; and gamma-MSH.

- 20 Morphiceptin peptides including, but not limited to, morphiceptin (beta-casomorphin 1-4 amide); [D-Pro⁴]-morphiceptin; and [N-MePhe³,D-Pro⁴]-morphiceptin.

Motilin peptides including, but not limited to, motilin, canine; motilin, porcine; biotinyl-motilin, porcine; and [Leu¹³]-motilin, porcine.

- 25 Neuro-peptides including, but not limited to, Ac-Asp-Glu; achatina cardio-excitatory peptide-1 (ACEP-1) (Achatina fulica); adipokinetic hormone (AKH) (Locust); adipokinetic hormone (Heliothis zea and Manduca sexta); alytesin; Tabanus atratus adipokinetic hormone (Taa-AKH); adipokinetic hormone II (Locusta migratoria); adipokinetic hormone II (Schistocerca gregaria); adipokinetic hormone III (AKH-3); adipokinetic hormone G (AKH-

G) (*Gryllus bimaculatus*); allatotropin (AT) (*Manduca sexta*); allatotropin 6-13 (*Manduca sexta*); APGW amide (*Lymnaea stagnalis*); buccalin; cerebellin; [Des-Ser¹]-cerebellin; corazonin (American Cockroach *Periplaneta americana*); crustacean cardioactive peptide (CCAP); crustacean erythrophore; DF2 (*Procambarus clarkii*); diazepam-binding inhibitor fragment, human; diazepam binding inhibitor fragment (ODN); eledoisin related peptide; FMRF amide (molluscan cardioexcitatory neuro-peptide); Gly-Pro-Glu (GPE), human; granuliberin R; head activator neuropeptide; [His⁷]-corazonin; stick insect hypertrehalosaemic factor II; *Tabanus atratus* hypotrehalosemic hormone (Taa-HoTH); isoguvacine hydrochloride; bicuculline methiodide; piperidine-4-sulphonic acid; joining peptide of proopiomelanocortin (POMC), bovine; joining peptide, rat; KSAYMRF amide (P. *redivivus*); kassinin; kinetensin; levitide; litorin; LUQ 81-91 (*Aplysia californica*); LUQ 83-91 (*Aplysia californica*); myoactive peptide I (Periplanetin CC-1) (Neuro-hormone D); myoactive peptide II (Periplanetin CC-2); myomodulin; neuron specific peptide; neuron specific enolase 404-443, rat; neuropeptide FF; neuropeptide K, porcine; NEI (prepro-MCH 131-143) neuropeptide, rat; NGE (prepro-MCH 110-128) neuropeptide, rat; NFL (*Procambarus clarkii*); PBAN-1 (*Bombyx mori*); Hez-PBAN (*Heliothis zea*); SCPB (cardioactive peptide from *aplysia*); secretoneurin, rat; uperolein; urechistachykinin I; urechistachykinin II; xenopsin-related peptide I; xenopsin-related peptide II; pedal peptide (Pep), *aplysia*; peptide Fl, lobster; phyllomedusin; polistes mastoparan; proctolin; ranatensin; Ro I (Lubber Grasshopper, *Romalea microptera*); Ro II (Lubber Grasshopper, *Romalea microptera*); SALMF amide 1 (S1); SALMF amide 2 (S2); and SCPA.

Neuropeptide Y (NPY) peptides including, but not limited to, [Leu³¹,Pro³⁴]-neuropeptide Y, human; neuropeptide F (*Moniezia expansa*); B1BP3226 NPY antagonist; Bis (31/31') {[Cys³¹, Trp³², Nva³⁴] NPY 31-36}; neuropeptide Y, human, rat; neuropeptide Y 1-24 amide, human; biotinyl-neuropeptide Y; [D-Tyr^{27,36}, D-Thr³²]-NPY 27-36; Des 10-17 (cyclo 7-21) [Cys^{7,21}, Pro³⁴]-NPY; C2-NPY; [Leu³¹, Pro³⁴] neuropeptide Y, human; neuropeptide Y, free acid, human; neuropeptide Y, free acid, porcine; prepro NPY 68-97, human; N-acetyl-[Leu²⁸, Leu³¹] NPY 24-36; neuropeptide Y, porcine; [D-Trp³²]-neuropeptide Y, porcine; [D-Trp³²] NPY 1-36, human; [Leu¹⁷,DTrp³²] neuropeptide Y, human; [Leu³¹, Pro³⁴]-NPY, porcine; NPY 2-36, porcine; NPY 3-36, human; NPY 3-36, porcine; NPY 13-36, human; NPY 13-36, porcine; NPY 16-36, porcine; NPY 18-36, porcine; NPY 20-36;

NFY 22-36; NPY 26-36; [Pro³⁴]-NPY 1-36, human; [Pro³⁴]-neuropeptide Y, porcine; PYX-1; PYX-2; T4-[NPY(33-36)]4; and Tyr(OMe)²¹]-neuropeptide Y, human.

Neurotropic factor peptides including, but not limited to, glial derived neurotropic factor (GDNF); brain derived neurotropic factor (BDNF); and ciliary neurotropic factor (CNTF).

Orexin peptides including, but not limited to, orexin A; orexin B, human; orexin B, rat, mouse.

Opioid peptides including, but not limited to, alpha-casein fragment 90-95; BAM-18P; casomokinin L; casoxin D; crystalline; DALDA; dermenkephalin (deltorphin) (Phylomedusa sauvagei); [D-Ala²]-deltorphin I; [D-Ala²]-deltorphin II; endomorphin-1; endomorphin-2; kyotorphin; [DArg²]-kyotorphin; morphin tolerance peptide; morphine modulating peptide, C-terminal fragment; morphine modulating neuropeptide (A-18-F-NH2); nociceptin [orphanin FQ] (ORL1 agonist); TIPP; Tyr-MIF-1; Tyr-W-MIF-1; valorphin; LW-hemorphin-6, human; Leu-valorphin-Arg; and Z-Pro-D-Leu.

Oxytocin peptides including, but not limited to, [Asu⁶]-oxytocin; oxytocin; biotinyl-oxytocin; [Thr⁴, Gly⁷]-oxytocin; and tocinoic acid ([Ile³]-pressinoic acid).

PACAP (pituitary adenylating cyclase activating peptide) peptides including, but not limited to, PACAP 1-27, human, ovine, rat; PACAP (1-27)-Gly-Lys-Arg-NH2, human; [Des-Gln¹⁶]-PACAP 6-27, human, ovine, rat; PACAP38, frog; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP38 16-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP-related peptide (PRP), human; and PACAP-related peptide (PRP), rat.

Pancreastatin peptides including, but not limited to, chromostatin, bovine; pancreastatin (hPST-52) (chromogranin A 250-301, amide); pancreastatin 24-52 (hPST-29), human; chromogranin A 286-301, amide, human; pancreastatin, porcine; biotinyl-pancreastatin, porcine; [Nle⁸]-pancreastatin, porcine; [Tyr⁰,Nle⁸]-pancreastatin, porcine; [Tyr⁰]-pancreastatin, porcine; parastatin 1-19 (chromogranin A 347-365), porcine; pancreastatin (chromogranin A 264-314-amide, rat; biotinyl-pancreastatin (biotinyl-

chromogranin A 264-314-amide; [Tyr⁰]-pancreastatin, rat; pancreastatin 26-51, rat; and pancreastatin 33-49, porcine.

Pancreatic polypeptides including, but not limited to, pancreatic polypeptide, avian; pancreatic polypeptide, human; C-fragment pancreatic polypeptide acid, human; C-fragment 5 pancreatic polypeptide amide, human; pancreatic polypeptide (*Rana temporaria*); pancreatic polypeptide, rat; and pancreatic polypeptide, salmon.

Parathyroid hormone peptides including, but not limited to, [Asp⁷⁶]-parathyroid hormone 39-84, human; [Asp⁷⁶]-parathyroid hormone 53-84, human; [Asn⁷⁶]-parathyroid hormone 1-84, human; [Asn⁷⁶]-parathyroid hormone 64-84, human; [Asn⁸, Leu¹⁸]-parathyroid hormone 1-34, human; [Cys^{5,28}]-parathyroid hormone 1-34, human; hypercalcemia malignancy factor 1-40; [Leu¹⁸]-parathyroid hormone 1-34, human; [Lys(biotinyl)¹³, Nle^{8,18}, Tyr³⁴]-parathyroid hormone 1-34 amide; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 1-34 amide; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 3-34 amide, bovine; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 1-34, human; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 1-34 amide, human; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 3-34 amide, human; [Nle^{8,18}, Tyr³⁴]-parathyroid hormone 7-34 amide, bovine; [Nle^{8,21}, Tyr³⁴]-parathyroid hormone 1-34 amide, rat; parathyroid hormone 44-68, human; parathyroid hormone 1-34, bovine; parathyroid hormone 3-34, bovine; parathyroid hormone 1-31 amide, human; parathyroid hormone 1-34, human; parathyroid hormone 13-34, human; parathyroid hormone 1-34, rat; parathyroid hormone 1-20 38, human; parathyroid hormone 1-44, human; parathyroid hormone 28-48, human; parathyroid hormone 39-68, human; parathyroid hormone 39-84, human; parathyroid hormone 53-84, human; parathyroid hormone 69-84, human; parathyroid hormone 70-84, human; [Pro³⁴]-peptide YY (PYY), human; [Tyr⁰]-hypercalcemia malignancy factor 1-40; [Tyr⁰]-parathyroid hormone 1-44, human; [Tyr⁰]-parathyroid hormone 1-34, human; [Tyr¹]-parathyroid hormone 1-34, human; [Tyr²⁷]-parathyroid hormone 27-48, human; [Tyr³⁴]-parathyroid hormone 7-34 amide, bovine; [Tyr⁴³]-parathyroid hormone 43-68, human; [Tyr⁵², Asn⁷⁶]-parathyroid hormone 52-84, human; and [Tyr⁶³]-parathyroid hormone 63-84, human.

Parathyroid hormone (PTH)-related peptides including, but not limited to, PTHrP ([Tyr³⁶]-PTHrP 1-36 amide), chicken; hHCF-(1-34)-NH₂ (humoral hypercalcemic factor), 30 human; PTH-related protein 1-34, human; biotinyl-PTH-related protein 1-34, human; [Tyr⁰]-PTH-related protein 1-34, human; [Tyr³⁴]-PTH-related protein 1-34 amide, human; PTH-related protein 1-37, human; PTH-related protein 7-34 amide, human; PTH-related protein

38-64 amide, human; PTH-related protein 67-86 amide, human; PTH-related protein 107-111, human, rat, mouse; PTH-related protein 107-111 free acid; PTH-related protein 107-138, human; and PTH-related protein 109-111, human.

5 Peptide T peptides including, but not limited to, peptide T; [D-Ala¹]-peptide T; and [D-Ala¹]-peptide T amide.

Prolactin-releasing peptides including, but not limited to, prolactin-releasing peptide 31, human; prolactin-releasing peptide 20, human; prolactin-releasing peptide 31, rat; prolactin-releasing peptide 20, rat; prolactin-releasing peptide 31, bovine; and prolactin-releasing peptide 20, bovine.

10 Peptide YY (PYY) peptides including, but not limited to, PYY, human; PYY 3-36, human; biotinyl-PYY, human; PYY, porcine, rat; and [Leu³¹, Pro³⁴]-PYY, human.

15 Renin substrate peptides including, but not limited to, acetyl, angiotensinogen 1-14, human; angiotensinogen 1-14, porcine; renin substrate tetradecapeptide, rat; [Cys⁸]-renin substrate tetradecapeptide, rat; [Leu⁸]-renin substrate tetradecapeptide, rat; and [Val⁸]-renin substrate tetradecapeptide, rat.

Secretin peptides including, but not limited to, secretin, canine; secretin, chicken; secretin, human; biotinyl-secretin, human; secretin, porcine; and secretin, rat.

20 Somatostatin (GIF) peptides including, but not limited to, BIM-23027; biotinyl-somatostatin; biotinylated cortistatin 17, human; cortistatin 14, rat; cortistatin 17, human; [Tyr⁰]-cortistatin 17, human; cortistatin 29, rat; [D-Trp⁸]-somatostatin; [DTrp⁸, DCys¹⁴]-somatostatin; [DTrp⁸, Tyr¹¹]-somatostatin; [D-Trp¹¹]-somatostatin; NTB (Naltriben); [Nle⁸]-somatostatin 1-28; octreotide (SMS 201-995); prosomatostatin 1-32, porcine; [Tyr⁰]-somatostatin; [Tyr¹]-somatostatin; [Tyr¹]-somatostatin 28 (1-14); [Tyr¹¹]-somatostatin; [Tyr⁰, D-Trp⁸]-somatostatin; somatostatin; somatostatin antagonist; somatostatin-25; somatostatin-28; somatostatin 28 (1-12); biotinyl-somatostatin-28; [Tyr⁰]-somatostatin-28; [Leu⁸, D-Trp²², Tyr²⁵]-somatostatin-28; biotinyl-[Leu⁸, D-Trp²², Tyr²⁵]-somatostatin-28; somatostatin-28 (1-14); and somatostatin analog, RC-160.

25 Substance P peptides including, but not limited to, G protein antagonist-2; Ac-[Arg⁶, Sar⁹, Met(O2)¹¹]-substance P 6-11; [Arg³]-substance P; Ac-Trp-3,5-bis(trifluoromethyl)benzyl ester; Ac-[Arg⁶, Sar⁹, Met(O2)¹¹]-substance P 6-11; [D-Ala⁴]-substance P 4-11; [Tyr⁶, D-Phe⁷, D-His⁹]-substance P 6-11 (sendide); biotinyl-substance P; biotinyl-NTE[Arg³]-substance P; [Tyr⁸]-substance P; [Sar⁹, Met(O2)¹¹]-substance P; [D-Pro², D-Trp^{7,9}]-substance

P; [D-Pro⁴, 0-Trp^{7,9}]-substance P 4-11; substance P 4-11; [DTrp^{2,7,9}]-substance P; [(Dehydro)Pro^{2,4}, Pro⁹]-substance P; [Dehydro-Pro⁴]-substance P 4-11; [Glp⁵-(Me)Phe⁸, Sar⁹]-substance P 5-11; [Glp⁵, Sar⁹]-substance P 5-11; [Glp⁵]-substance P 5-11; hepta-substance P (substance P 5-11); hexa-substance P(substance P 6-11); [MePhe⁸, Sar⁹]-substance P; [Nle¹¹]-substance P; Octa-substance P(substance P 4-11); [pGlu¹]-hexa-substance P ([pGlu⁶]-substance P 6-11); [pGlu⁶, D-Pro⁹]-substance P 6-11; [(pNO₂)Phe⁷Nle¹¹]-substance P; penta-substance P (substance P 7-11); [Pro⁹]-substance P; GR73632, substance P 7-11; [Sar⁴]-substance P 4-11; [Sar⁹]-substance P; peptide ([pGlu⁶, Pro⁹]-substance P 6-11); spantide I; spantide II; substance P; substance P, cod; substance P, trout; substance P antagonist; substance P-Gly-Lys-Arg; substance P 1-4; substance P 1-6; substance P 1-7; substance P 1-9; deca-substance P (substance P 2-11); nona-substance P (substance P 3-11); substance P tetrapeptide (substance P 8-11); substance P tripeptide (substance P 9-11); substance P, free acid; substance P methyl ester; and [Tyr⁸, Nle¹¹] substance P.

Tachykinin peptides including, but not limited to, [Ala⁵, beta-Ala⁸] neurokinin A 4-10; eledoisin; locustatachykinin I (Lom-TK-I) (*Locusta migratoria*); locustatachykinin II (Lom-TK-II) (*Locusta migratoria*); neurokinin A 4-10; neurokinin A (neuromedin L, substance K); neurokinin A, cod and trout; biotinyl-neurokinin A (biotinyl-neuromedin L, biotinyl-substance K); [Tyr⁰]-neurokinin A; [Tyr⁶]-substance K; FR64349; [Lys³, Gly⁸-(R)-gamma-lactam-Leu⁹]-neurokinin A 3-10; GR83074; GR87389; GR94800; [Beta-Ala⁸]-neurokinin A 4-10; [Nle¹⁰]-neurokinin A 4-10; [Trp⁷, beta-Ala⁸]-neurokinin A 4-10; neurokinin B (neuromedin K); biotinyl-neurokinin B (biotinyl-neuromedin K); [MePhe⁷]-neurokinin B; [Pro⁷]-neurokinin B; [Tyr⁰]-neurokinin B; neuromedin B, porcine; biotinyl-neuromedin B, porcine; neuromedin B-30, porcine; neuromedin B-32, porcine; neuromedin B receptor antagonist; neuromedin C, porcine; neuromedin N, porcine; neuromedin (U-8), porcine; neuromedin (U-25), porcine; neuromedin U, rat; neuropeptide-gamma (gamma-preprotachykinin 72-92); PG-KII; phyllolitorin; [Leu⁸]-phyllolitorin (*Phyllomedusa sauvagei*); physalaemin; physalaemin 1-11; scyliorhinin II, amide, dogfish; senktide, selective neurokinin B receptor peptide; [Ser²]-neuromedin C; beta-preprotachykinin 69-91, human; beta-preprotachykinin 111-129, human; tachyplesin I; xenopsin; and xenopsin 25 (xenin 25), human.

Thyrotropin-releasing hormone (TRH) peptides including, but not limited to, biotinyl-thyrotropin-releasing hormone; [Glu¹]-TRH; His-Pro-diketopiperazine; [3-Me-His²]-TRH;

pGlu-Gln-Pro-amide; pGlu-His; [Phe²]-TRH; prepro TRH 53-74; prepro TRH 83-106; prepro-TRH 160-169 (Ps4, TRH-potentiating peptide); prepro-TRH 178-199; thyrotropin-releasing hormone (TRH); TRH, free acid; TRH-SH Pro; and TRH precursor peptide.

Toxin peptides including, but not limited to, omega-agatoxin TK; agelenin, (spider,

5 Agelena opulenta); apamin (honeybee, *Apis mellifera*); calcicudine (CaC) (green mamba, *Dendroaspis angusticeps*); calciseptine (black mamba, *Dendroaspis polylepis polylepis*); charybdotoxin (ChTX) (scorpion, *Leiurus quinquestriatus* var. *hebraeus*); chlorotoxin; conotoxin GI (marine snail, *Conus geographus*); conotoxin GS (marine snail, *Conus geographus*); conotoxin MI (Marine *Conus magus*); alpha-conotoxin EI, *Conus ermineus*; 10 alpha-conotoxin SIA; alpha-conotoxin Iml; alpha-conotoxin SI (cone snail, *Conus striatus*); micro-conotoxin GIIIB (marine snail, *Conus geographus*); omega-conotoxin GVIA (marine snail, *Conus geographus*); omega-conotoxin MVIIA (*Conus magus*); omega-conotoxin MVIIC (*Conus magus*); omega-conotoxin SVIB (cone snail, *Conus striatus*); endotoxin inhibitor; geographutoxin I (GTx-I) (μ -Conotoxin GIIIA); iberiotoxin (IbTX) (scorpion, *Buthus tamulus*); kaliotoxin 1-37; kaliotoxin (scorpion, *Androctonus mauretanicus* mauretanicus); mast cell-degranulating peptide (MCD-peptide, peptide 401); margatoxin (MgTX) (scorpion, *Centruroides Margaritatus*); neurotoxin NSTX-3 (pupa new guinean spider, *Nephilia maculata*); PLTX-II (spider, *Plectreurys tristes*); scyllatoxin (leiurotoxin I); and stichodactyla toxin (ShK).

20 Vasoactive intestinal peptides (VIP/PHI) including, but not limited to, VIP, human, porcine, rat, ovine; VIP-Gly-Lys-Arg-NH2; biotinyl-PHI (biotinyl-PHI-27), porcine; [Glp¹⁶] VIP 16-28, porcine; PHI (PHI-27), porcine; PHI (PHI-27), rat; PHM-27 (PHI), human; prepro VIP 81-122, human; preproVIP/PHM 111-122; prepro VIP/PHM 156-170; biotinyl-25 PHM-27 (biotinyl-PHI), human; vasoactive intestinal contractor (endothelin-beta); vasoactive intestinal octacosa-peptide, chicken; vasoactive intestinal peptide, guinea pig; biotinyl-VIP, human, porcine, rat; vasoactive intestinal peptide 1-12, human, porcine, rat; vasoactive intestinal peptide 10-28, human, porcine, rat; vasoactive intestinal peptide 11-28, human, porcine, rat, ovine; vasoactive intestinal peptide (cod, *Gadus morhua*); vasoactive intestinal peptide 6-28; vasoactive intestinal peptide antagonist; vasoactive intestinal peptide antagonist 30 ([Ac-Tyr¹, D-Phe²]-GHRF 1-29 amide); vasoactive intestinal peptide receptor antagonist (4-Cl-D-Phe⁶, Leu¹⁷]-VIP); and vasoactive intestinal peptide receptor binding inhibitor, L-8-K.

Vasopressin (ADH) peptides including, but not limited to, vasopressin; [Asu^{1,6},Arg⁸]-vasopressin; vasotocin; [Asu^{1,6},Arg⁸]-vasotocin; [Lys⁸]-vasopressin; pressinoic acid; [Arg⁸]-desamino vasopressin desglycinamide; [Arg⁸]-vasopressin (AVP); [Arg⁸]-vasopressin desglycinamide; biotinyl-[Arg⁸]-vasopressin (biotinyl-APP); [D-Arg⁸]-vasopressin; 5 desamino-[Arg⁸]-vasopressin; desamino-[D-Arg⁸]-vasopressin (DDAVP); [deamino-[D-3-(3'-pyridyl-Ala)]-[Arg⁸]-vasopressin; [1-(beta-Mercapto-beta, beta-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-[Arg⁸]-vasopressin; vasopressin metabolite neuropeptide [pGlu⁴, Cys⁶]; vasopressin metabolite neuropeptide [pGlu⁴, Cys⁶]; [Lys⁸]-deamino vasopressin desglycinamide; [Lys⁸]-vasopressin; [Mpr¹,Val⁴,DArg⁸]-vasopressin; 10 [Phe², Ile³, Orn⁸]-vasopressin ([Phe², Orn⁸]-vasotocin); [Arg⁸]-vasotocin; and [d(CH2)5, Tyr(Me)², Orn⁸]-vasotocin.

Virus related peptides including, but not limited to, fluorogenic human CMV protease substrate; HCV core protein 59-68; HCV NS4A protein 18-40 (JT strain); HCV NS4A protein 21-34 (JT strain); hepatitis B virus receptor binding fragment; hepatitis B virus pre-S region 120-145; [Ala¹²⁷]-hepatitis B virus pre-S region 120-131; herpes virus inhibitor 2; HIV envelope protein fragment 254-274; HIV gag fragment 129-135; HIV substrate; P 18 peptide; peptide T; [3,5 diiodo-Tyr⁷] peptide T; R15K HIV-1 inhibitory peptide; T20; T21; V3 decapeptide P 18-110; and virus replication inhibiting peptide.

While certain analogs, fragments, and/or analog fragments of the various polypeptides have been described above, it is to be understood that other analogs, fragments, and/or analog fragments that retain all or some of the activity of the particular polypeptide may also be useful in embodiments of the present invention. Analogs may be obtained by various means, as will be understood by those skilled in the art. For example, certain amino acids may be substituted for other amino acids in a polypeptide without appreciable loss of interactive 25 binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. As the interactive capacity and nature of a polypeptide drug defines its biological functional activity, certain amino acid sequence substitutions can be made in the amino acid sequence and nevertheless remain a polypeptide with like properties.

In making such substitutions, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive 30 biologic function on a polypeptide is generally understood in the art. It is accepted that the

relative hydrophobic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydrophobic index on the basis of its

5 hydrophobicity and charge characteristics as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). As will be understood by those skilled in the art, certain amino acids may be substituted by other amino acids having a similar hydrophobic index or score and still result in a polypeptide with similar biological activity, *i.e.*, still obtain a biological 10 functionally equivalent polypeptide. In making such changes, the substitution of amino acids whose hydrophobic indices are within ± 2 of each other is preferred, those which are within ± 1 of each other are particularly preferred, and those within ± 0.5 of each other are even more 15 particularly preferred.

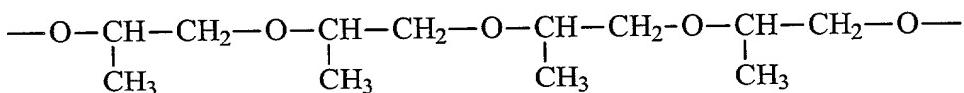
It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 provides that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 20 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (± 3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). As is understood by 25 those skilled in the art, an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 of each other is preferred, those which are within ± 1 of each other are particularly preferred, and those within ± 0.5 of each other are even more 30 particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their

hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions (i.e., amino acids that may be interchanged without significantly altering the biological activity of the polypeptide) that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include, for example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In embodiments of the present invention, a substantially monodispersed mixture of drug-oligomer conjugates is provided. Preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight. More preferably,, the mixture is a monodispersed mixture. Even more preferably, the mixture is a substantially purely monodispersed mixture of drug-oligomer conjugates. Still more preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight and the same molecular structure. Most preferably, the mixture is a purely monodispersed mixture.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butylidiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac₂O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butylidiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride (MeSO₂Cl) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B₁ de-blocking reagent to remove the blocking moiety B₁ and provide a primary alcohol extension monomer **57**. The B₁ de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B₁ de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B₁ de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B₁ de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to

provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono- or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the

primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B₁ blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

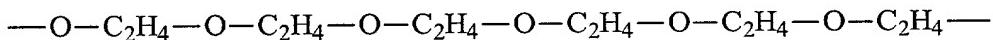
An end of a secondary alcohol extension mono- or poly-mer or an end of a secondary alcohol extension mono- or poly-mer mesylate may be reacted with a secondary alcohol capping mono- or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B₂ blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure**

12 show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

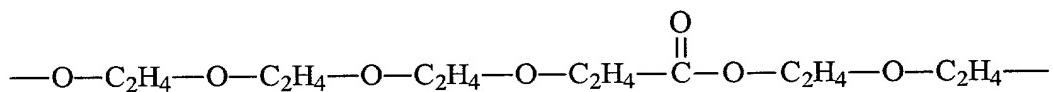
5 Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

10 The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

15 The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety



20 is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety



25 is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

30 The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched

fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a

carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood

5 that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellar or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately
10 functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug.

25 The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to
30 the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by

hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When 5 the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human 10 insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono- 15 conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys¹¹, Lys¹⁸ and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino 20 functionality of Lys¹¹ and an oligomer is coupled to the amino functionality of Lys¹⁸. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe¹, Lys³⁸, Lys⁴¹, Lys⁷⁰, Lys¹¹⁵, Lys¹⁴⁰, Lys¹⁴⁵, Lys¹⁵⁸, Lys¹⁶⁸, 25 and/or Lys¹⁷².

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Substantially monodispersed mixtures of drug-oligomer conjugates of the present invention may be synthesized by various methods. For example, a substantially monodispersed mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a substantially monodispersed mixture of carboxylic acid with a substantially monodispersed mixture of polyethylene glycol under conditions sufficient to provide a substantially monodispersed mixture of oligomers. The oligomers of the substantially monodispersed mixture are then activated so that they are capable of reacting

with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The substantially monodispersed mixture of activated oligomers may be reacted with a substantially monodispersed mixture of drugs under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the substantially monodispersed mixture of activated oligomers and the substantially monodispersed mixture of drugs is a substantially monodispersed mixture. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a substantially monodispersed mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be

understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC).

This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at one or more of the N-termini of the polypeptide. Following such blocking, the substantially monodispersed mixture of blocked drugs may be reacted with the substantially monodispersed mixture of activated oligomers to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a substantially monodispersed mixture of drug-oligomer conjugates. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

100-100-100-100-100

As another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography. The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a

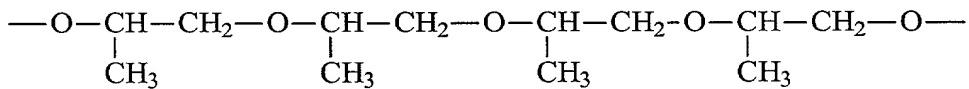
baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are 5 then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention preferably have two or more of the above-described 10 improved properties. More preferably, substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention have all four of the above-described improved properties.

In still other embodiments according to the present invention, a mixture of conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons is provided. Each conjugate in the mixture includes a drug coupled to an oligomer that comprises a polyalkylene glycol moiety. The standard deviation is preferably less than about 14 Daltons and is more preferably less than about 11 Daltons. The molecular weight distribution may be determined by methods known to those skilled in the art including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER 20 CHEMISTRY 394-402 (2d. ed., 1991). The standard deviation of the molecular weight distribution may then be determined by statistical methods as will be understood by those skilled in the art.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene 30 glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol

moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



- 5 This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.
- 10

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11 through 13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac₂O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride (MeSO₂Cl) to provide a primary extension alcohol monomer mesylate **55**.

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25 Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B₁ de-blocking reagent to remove the blocking moiety B₁ and provide a primary alcohol extension monomer **57**. The B₁ de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the

30

primary alcohol has been blocked by forming an ester, the B₁ de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B₁ de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B₁ de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B₂ de-blocking reagent to remove the blocking moiety B₂ and provide a secondary alcohol capping monomer **63**. The B₂ de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H₂ in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono- or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed

and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer 71.

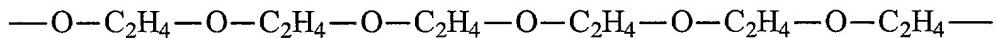
An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate 67 is reacted with the secondary alcohol capping monomer 63 to provide the capped/blocked primary alcohol extension trimer 68. The B₂ blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer 68. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

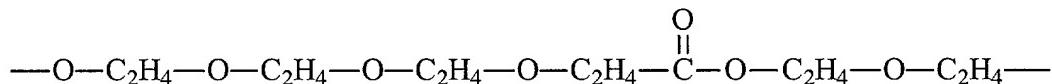
The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent

polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety



is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent

5 polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety



is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic
10 moiety having two polyethylene glycol subunits. Preferably, oligomers according to
embodiments of the present invention comprise a polyalkylene glycol moiety and do not
further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic
25 moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to
30 couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the 5 terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellar or liposomal encapsulation. According to embodiments of the 25 present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile 30 acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical

products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated 5 complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that 10 the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality 15 of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug 20 including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, 25 the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human 30 insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-

conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys¹¹, Lys¹⁸ and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys¹¹ and an oligomer is coupled to the amino functionality of Lys¹⁸. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe¹, Lys³⁸, Lys⁴¹, Lys⁷⁰, Lys¹¹⁵, Lys¹⁴⁰, Lys¹⁴⁵, Lys¹⁵⁸, Lys¹⁶⁸, and/or Lys¹⁷².

Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be synthesized by various methods. For example, a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a molecular weight distribution with a standard deviation of less than about 22 Daltons with a mixture of polyethylene glycol having a molecular weight distribution with a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The oligomers of the mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 6** and described in Examples 30-31.

hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be reacted with a mixture of drugs having a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the mixture of drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons is a mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a

human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

5 As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are 10 conjugated) having one or more oligomers at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be reacted with the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

15 Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less 20 than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average

weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

5 As another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of
10 less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography.

15 The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.
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As still another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates
25 having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to,
30 size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as
5 the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by
10 various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is
15 determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.
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Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have all four of the above-described improved properties.
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According to yet other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a drug coupled to an oligomer that comprises a
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polyalkylene glycol moiety, and the mixture has a dispersity coefficient (DC) greater than 10,000 where

$$DC = \frac{\left(\sum_{i=1}^n N_i M_i \right)^2}{\sum_{i=1}^n N_i M_i^2 - \sum_{i=1}^n N_i - \left(\sum_{i=1}^n N_i M_i \right)^2}$$

wherein:

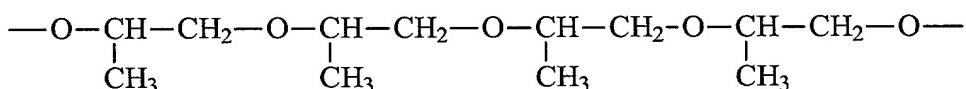
5 n is the number of different molecules in the sample;

N_i is the number of ith molecules in the sample; and

M_i is the mass of the ith molecule.

The mixture of conjugates preferably has a dispersity coefficient greater than 100,000. More preferably, the dispersity coefficient of the conjugate mixture is greater than 500,000 and, most preferably, the dispersity coefficient is greater than 10,000,000. The variables n, N_i, and M_i may be determined by various methods as will be understood by those skilled in the art, including, but not limited to, methods described below in Example 123.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety

of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is 5 preferably synthesized as illustrated in **Figures 11 through 13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-
10 butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac₂O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride (MeSO₂Cl) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B₁ de-blocking reagent to remove the blocking moiety B₁ and provide a primary alcohol extension monomer **57**. The B₁ de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B₁ de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol 25 has been blocked using a silylchloride, the B₁ de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension 30 monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not

limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**.

The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**.

5 The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-

10 blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol

capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

15 In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

20 For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**.

25 Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1

blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**.

30 Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69**

may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the 5 primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 10 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B₁ blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

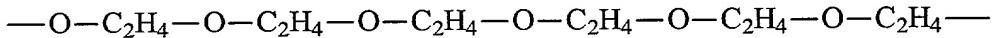
An end of a secondary alcohol extension mono- or poly-mer or an end of a secondary alcohol extension mono- or poly-mer mesylate may be reacted with a secondary alcohol capping mono- or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B₂ blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in

Figure 12 show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

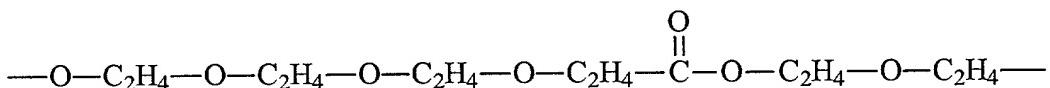
Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety



is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety



is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to

embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive

(i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other

5 embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood
10 that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellar or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to
15 impart hydrogen bonding capability), as will be understood by those skilled in the art.
According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting
20 oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under
25 suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug.
30 The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of

oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys¹¹, Lys¹⁸ and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys¹¹ and an oligomer is coupled to the amino functionality of Lys¹⁸. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe¹, Lys³⁸, Lys⁴¹, Lys⁷⁰, Lys¹¹⁵, Lys¹⁴⁰, Lys¹⁴⁵, Lys¹⁵⁸, Lys¹⁶⁸, and/or Lys¹⁷².

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 may be synthesized by various methods. For example, a mixture of oligomers having

a dispersity coefficient greater than 10,000 consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a dispersity coefficient greater than 10,000 with a mixture of polyethylene glycol having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of oligomers

5 having a dispersity coefficient greater than 10,000. The oligomers of the mixture having a dispersity coefficient greater than 10,000 are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another

10 embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis

15 route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route

20 for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 10** and described in Example

25 40 hereinbelow.

The mixture of activated oligomers having a dispersity coefficient greater than 10,000 is reacted with a mixture of drugs having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for

30 example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of

the mixture of activated oligomers having a dispersity coefficient greater than 10,000 and the mixture of drugs having a dispersity coefficient greater than 10,000 is a mixture having a dispersity coefficient greater than 10,000. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution

5 below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a dispersity coefficient greater than 10,000. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing

10 various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1} Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or

15 endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs having a dispersity coefficient greater than 10,000 may be reacted with the mixture of activated oligomers having a dispersity coefficient greater than 10,000 to provide a mixture of drug-oligomer conjugates having oligomer(s)

20 coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. Alternatively,

25 the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have improved

properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of
5 drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average molecular weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation
10 chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.
15
20

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the
25 rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a
30 polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average

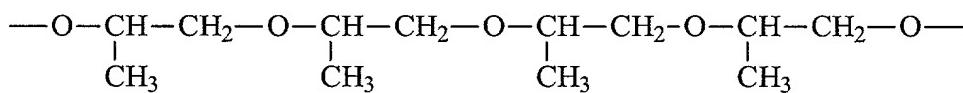
molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have all four of the above-described improved properties.

According to other embodiments of the present invention, a mixture of conjugates in which each conjugate includes a drug coupled to an oligomer and has the same number of polyalkylene glycol subunits is provided.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is
preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described.
As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking
reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking
reagent may be various primary alcohol blocking reagents as will be understood by those
skilled in the art including, but not limited to, silylchloride compounds such as t-
butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such
as Ac₂O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking
reagent that is substantially non-reactive with secondary alcohols, such as t-
butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension
monomer (**54**) may be reacted with methanesulfonyl chloride (MeSO₂Cl) to provide a
primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56**
5 may be reacted with a B₁ de-blocking reagent to remove the blocking moiety B₁ and provide a primary alcohol extension monomer **57**. The B₁ de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B₁ de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol
10 has been blocked using a silylchloride, the B₁ de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B₁ de-blocking agent as described above to provide a primary alcohol capping monomer **60**.
15 The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B₂ de-blocking reagent to remove the blocking moiety B₂ and provide a
20 secondary alcohol capping monomer **63**. The B₂ de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H₂ in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the
25 synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono- or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**.
10 Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be
25 understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.
30

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in

5 **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

10 An end of a secondary alcohol extension mono- or poly-mer or an end of a secondary alcohol extension mono- or poly-mer mesylate may be reacted with a secondary alcohol capping mono- or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in

15 **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in

20 **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly

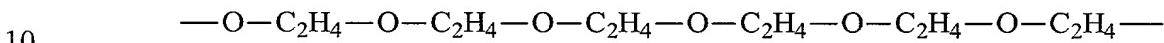
25 (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art

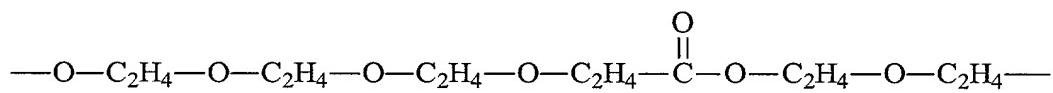
30 including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

5 The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety



is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety



is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

20 The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the 25 alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

30 The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic

moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

5 The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

10 The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

15 The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it

20 is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

25 While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellar or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately

functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art.

According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile

5 acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the

10 present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

15 More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by

20 hydrolysis in the body.

25

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for

30 example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human

insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human 5 insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the 10 amino functionality of Lys¹¹, Lys¹⁸ and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys¹¹ and an oligomer is coupled to the amino functionality of Lys¹⁸. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to 15 an amino functionality of Phe¹, Lys³⁸, Lys⁴¹, Lys⁷⁰, Lys¹¹⁵, Lys¹⁴⁰, Lys¹⁴⁵, Lys¹⁵⁸, Lys¹⁶⁸, and/or Lys¹⁷².

Mixtures of drug oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol where each oligomer in the mixture has the same number of polyethylene glycol subunits is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol where each polyethylene glycol molecule in the mixture has the same number of polyethylene glycol subunits under conditions sufficient to provide a mixture of oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits. 20 The oligomers of the mixture where each oligomer in the mixture has the same number of polyethylene glycol subunits are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 3** and described in Examples 25 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still 30

another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each 5 oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a 10 mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer in the mixture has the same 20 number of polyethylene glycol subunits is reacted with a mixture of drugs under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of 25 activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits and the mixture of drugs is a mixture of conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may 30 be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number of polyethylene glycol subunits. The degree of conjugation (e.g.,

whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, Lys^{B29} of a 5 human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on 10 the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired 15 to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one 20 or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to 25 de-blocking.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the 30 same number of polyethylene glycol subunits according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of

POLY(25)
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polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

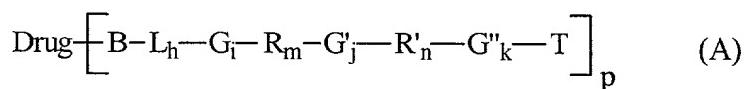
As still another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed

mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention have all four of the above-described improved properties.

According to still other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate has the same molecular weight and has the formula:



wherein:

B is a bonding moiety;

L is a linker moiety;

5 G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

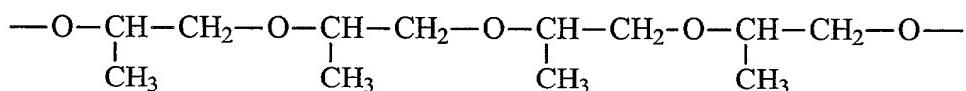
T is a terminating moiety;

h, i, j, k, m and n are individually 0 or 1, with the proviso that when R is the polyalkylene

10 glycol moiety; m is 1, and when R' is the polyalkylene glycol moiety, n is 1; and

p is an integer from 1 to the number of nucleophilic residues on the drug.

According to these embodiments of the present invention, R or R' is a polyalkylene moiety. The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



This uniform polypropylene glycol structure may be described as having only one methyl

25 substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties (i.e., the sum of m + n is 1). Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the

drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11 through 13**, which will now be described.

- 5 As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butylidiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac₂O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butylidiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride (MeSO₂Cl) to provide a primary extension alcohol monomer mesylate **55**.

10
15 Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B₁ de-blocking reagent to remove the blocking moiety B₁ and provide a primary alcohol extension monomer **57**. The B₁ de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B₁ de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B₁ de-blocking reagent is preferably
20 tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

25
30 The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B₁

de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B₁ blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono- or poly-mer or an end of a secondary alcohol extension mono- or poly-mer mesylate may be reacted with a secondary alcohol capping mono- or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B₂ blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a

uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly 5 (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art 10 including, but not limited to, those described herein with respect to polyethylene glycol moieties.

According to these embodiments of the present invention, R or R' is a lipophilic moiety as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most 20 preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

According to these embodiments of the present invention, the spacer moieties, G, G' and G", are spacer moieties as will be understood by those skilled in the art. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties. Preferably, oligomers of these embodiments do not include spacer moieties (i.e., i, j and k are 25 preferably 0).

According to these embodiments of the present invention, the linker moiety, L, may be used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid.

According to these embodiments of the present invention, the terminating moiety is 30 preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the

terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

According to these embodiments of the present invention, the oligomer, which is represented by the bracketed portion of the structure of Formula A, is covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. The bonding moiety, B, may be various bonding moieties that may be used to covalently couple the oligomer with the drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties.

The variable p is an integer from 1 to the number of nucleophilic residues on the drug. When p is greater than 1, more than one oligomer (i.e., a plurality of oligomers) is coupled to the drug. According to these embodiments of the present invention, the oligomers in the plurality are the same.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of

Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys¹¹, Lys¹⁸ and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys¹¹ and an oligomer is coupled to the amino functionality of Lys¹⁸. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe¹, Lys³⁸, Lys⁴¹, Lys⁷⁰, Lys¹¹⁵, Lys¹⁴⁰, Lys¹⁴⁵, Lys¹⁵⁸, Lys¹⁶⁸, and/or Lys¹⁷².

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol under conditions sufficient to provide a mixture of oligomers. The oligomers of the mixture are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 6** and described in Examples 30-31.

hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is reacted with a mixture of drugs where each drug in the mixture has the same molecular weight under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A and the mixture of drugs is a mixture of conjugates where each conjugate has the same molecular weight and has the structure Formula A. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number molecular weight and has the structure of Formula A. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or

verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on

5 the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following
10 such blocking, the mixture of blocked drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same molecular weight and has a structure of the oligomer of Formula A to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If
15 necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.
20

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A
25 preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates
30 where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel

permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has
5 an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each
10 conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured
15 using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the
20 structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including,
25 but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed

mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates
5 where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area
10 under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.
15

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have all four of the above-described improved properties.

Pharmaceutical compositions comprising a conjugate mixture according to embodiments of the present invention are also provided. The mixtures of drug-oligomer conjugates described above may be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.*, Remington, *The Science And Practice of Pharmacy* (9th Ed. 1995). In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the mixture of drug-oligomer conjugates is typically

admixed with, *inter alia*, a pharmaceutically acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the pharmaceutical composition and should not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the mixture of drug-oligomer conjugates as a unit-dose formulation, for example, a tablet, which may contain from about 5 0.01 or 0.5% to about 95% or 99% by weight of the mixture of drug-oligomer conjugates. The pharmaceutical compositions may be prepared by any of the well known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients.

10 The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, intracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal 15 administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular mixture of drug-oligomer conjugates which is being used.

20 Pharmaceutical compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tables, each containing a predetermined amount of the mixture of drug-oligomer conjugates; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the mixture of drug-oligomer conjugates and a suitable carrier (which may contain one or more accessory ingredients as noted above). In 25 general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the mixture of drug-oligomer conjugates with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the mixture of drug-oligomer conjugates, optionally with one 30 or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the mixture in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s).

Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the mixture of drug-oligomer conjugates in a flavoured base, usually 5 sucrose and acacia or tragacanth; and pastilles comprising the mixture of drug-oligomer conjugates in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions according to embodiments of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the mixture of drug-oligomer conjugates, which preparations are preferably 10 isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit\ dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for 15 example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition comprising a mixture of drug-oligomer conjugates in a unit dosage form in a sealed container may be provided. The mixture of drug-oligomer conjugates is provided in the form of a lyophilizate 20 which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the mixture of drug-oligomer conjugates. When the mixture of drug-oligomer conjugates is substantially water-insoluble, a 25 sufficient amount of emulsifying agent which is physiologically acceptable may be employed in sufficient quantity to emulsify the mixture of drug-oligomer conjugates in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably 30 presented as unit dose suppositories. These may be prepared by admixing the mixture of drug-oligomer conjugates with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

5 Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Compositions suitable for transdermal administration may also be delivered by iontophoresis (*see*, for example, *Pharmaceutical Research* 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous
10 solution of the mixture of drug-oligomer conjugates. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

Methods of treating an insulin deficiency in a subject in need of such treatment by administering an effective amount of such pharmaceutical compositions are also provided. The effective amount of any mixture of drug-oligomer conjugates, the use of which is in the scope of present invention, will vary somewhat from mixture to mixture, and patient to patient, and will depend upon factors such as the age and condition of the patient and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the mixture of drug-oligomer conjugates. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base.
20 A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of insulin deficiency being treated and may be for as long as the life of the patient.

Methods of synthesizing conjugate mixtures according to embodiments of the present invention are also provided. While the following embodiments of a synthesis route are directed to synthesis of a substantially monodispersed mixture, similar synthesis routes may

be utilized for synthesizing other drug-oligomer conjugate mixtures according to embodiments of the present invention.

A substantially monodispersed mixture of polymers comprising polyethylene glycol moieties is provided as illustrated in reaction 1:



5 (I) (II) (III)

R¹ is H or a lipophilic moiety. R¹ is preferably H, alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, an ester of a fatty acid moiety, cholesteryl, or adamantyl. R¹ is more preferably H, lower alkyl, or an aromatic moiety. R¹ is most preferably H, methyl, or benzyl.

10 In Formula I, n is from 1 to 25. Preferably n is from 1 to 6.

X⁺ is a positive ion. Preferably X⁺ is any positive ion in a compound, such as a strong base, that is capable of ionizing a hydroxyl moiety on PEG. Examples of positive ions include, but are not limited to, sodium ions, potassium ions, lithium ions, cesium ions, and thallium ions.

15 R² is H or a lipophilic moiety. R² is preferably linear or branched alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, or an ester of a fatty acid moiety. R² is more preferably lower alkyl, benzyl, a fatty acid moiety having 1 to 24 carbon atoms, or an ester of a fatty acid moiety having 1 to 24 carbon atoms. R² is most preferably methyl, a fatty acid moiety having 1 to 18 carbon atoms or an ethyl ester of a fatty acid moiety having 1 to 18 carbon atoms.

20 In Formula II, m is from 1 to 25. Preferably m is from 1 to 6.

Ms is a mesylate moiety (i.e., CH₃S(O₂)⁻).

As illustrated in reaction 1, a mixture of compounds having the structure of Formula I is reacted with a mixture of compounds having the structure of Formula II to provide a mixture of polymers comprising polyethylene glycol moieties and having the structure of Formula III. The mixture of compounds having the structure of Formula I is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula I have the same molecular weight, and, more preferably, the mixture of compounds of Formula I is a monodispersed mixture. The mixture of compounds of Formula II is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula II

have the same molecular weight, and, more preferably, the mixture of compounds of Formula II is a monodispersed mixture. The mixture of compounds of Formula III is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compound of Formula III have the same molecular weight. More 5 preferably, the mixture of compounds of Formula III is a monodispersed mixture.

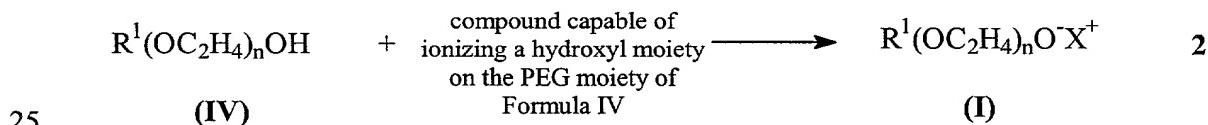
Reaction **1** is preferably performed between about 0°C and about 40°C, is more preferably performed between about 15°C and about 35°C, and is most preferably performed at room temperature (approximately 25°C).

Reaction 1 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 1 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction 1 is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrrolidinone, tetrahydronaphthalene, decahydronaphthalene, 1,2-dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, DMA or toluene.

The molar ratio of the compound of Formula I to the compound of Formula II is preferably greater than about 1:1. More preferably, the molar ratio is at least about 2:1. By providing an excess of the compounds of Formula I, one can ensure that substantially all of the compounds of Formula II are reacted, which may aid in the recovery of the compounds of Formula III as discussed below.

Compounds of Formula I are preferably prepared as illustrated in reaction 2:



R^1 and X^+ are as described above and the mixture of compounds of Formula IV is substantially monodispersed; preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula IV have the same molecular weight; and, more preferably, the mixture of compounds of Formula IV is a monodispersed mixture.

30 Various compounds capable of ionizing a hydroxyl moiety on the PEG moiety of the

compound of Formula IV will be understood by those skilled in the art. The compound capable of ionizing a hydroxyl moiety is preferably a strong base. More preferably, the compound capable of ionizing a hydroxyl moiety is selected from the group consisting of sodium hydride, potassium hydride, sodium t-butoxide, potassium t-butoxide, butyl lithium 5 (BuLi), and lithium diisopropylamine. The compound capable of ionizing a hydroxyl moiety is more preferably sodium hydride.

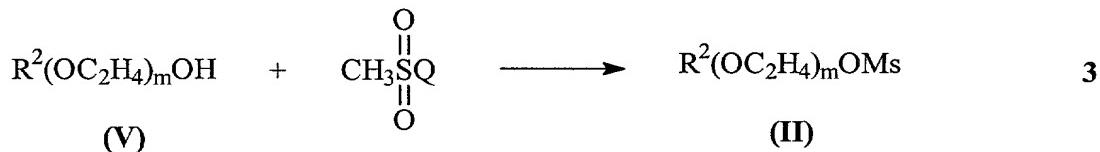
The molar ratio of the compound capable of ionizing a hydroxyl moiety on the PEG moiety of the compound of Formula IV to the compound of Formula IV is preferably at least about 1:1, and is more preferably at least about 2:1. By providing an excess of the compound 10 capable of ionizing the hydroxyl moiety, it is assured that substantially all of the compounds of Formula IV are reacted to provide the compounds of Formula I. Thus, separation difficulties, which may occur if both compounds of Formula IV and compounds of Formula I were present in the reaction product mixture, may be avoided.

Reaction 2 is preferably performed between about 0°C and about 40°C, is more 15 preferably performed between about 0°C and about 35°C, and is most preferably performed between about 0°C and room temperature (approximately 25°C).

Reaction 2 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 2 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction 2 is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrrolidinone, dichloromethane, chloroform, tetrahydronaphthalene, decahydronaphthalene, 1,2- 25 dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, dichloromethane or toluene.

Compounds of Formula II are preferably prepared as illustrated in reaction 3:



R² and Ms are as described above and the compound of Formula V is present as a 30 substantially monodispersed mixture of compounds of Formula V; preferably at least about

96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula V have the same molecular weight; and, more preferably, the mixture of compounds of Formula V is a monodispersed mixture.

Q is a halide, preferably chloride or fluoride.

5 $\text{CH}_3\text{S}(\text{O}_2)\text{Q}$ is methanesulfonyl halide. The methanesulfonyl halide is preferably methanesulfonyl chloride or methanesulfonyl fluoride. More preferably, the methanesulfonyl halide is methanesulfonyl chloride.

The molar ratio of the methane sulfonyl halide to the compound of Formula V is preferably greater than about 1:1, and is more preferably at least about 2:1. By providing an 10 excess of the methane sulfonyl halide, it is assured that substantially all of the compounds of Formula V are reacted to provide the compounds of Formula II. Thus, separation difficulties, which may occur if both compounds of Formula V and compounds of Formula II were present in the reaction product mixture, may be avoided.

15 Reaction 3 is preferably performed between about -10°C and about 40°C, is more preferably performed between about 0°C and about 35°C, and is most preferably performed between about 0°C and room temperature (approximately 25°C).

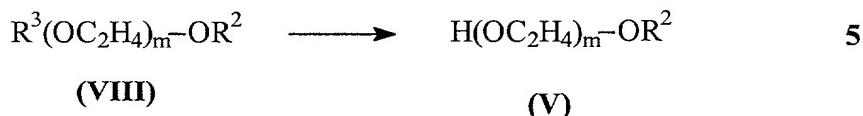
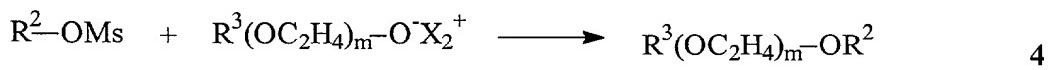
Reaction 3 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 3 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

20 Reaction 3 is preferably carried out in the presence of an aliphatic amine including, but not limited to, monomethylamine, dimethylamine, trimethylamine, monoethylamine, diethylamine, triethylamine, monoisopropylamine, diisopropylamine, mono-n-butylamine, di-n-butylamine, tri-n-butylamine, monocyclohexylamine, dicyclohexylamine, or mixtures thereof. More preferably, the aliphatic amine is a tertiary amine such as triethylamine.

25 As will be understood by those skilled in the art, various substantially monodispersed mixtures of compounds of Formula V are commercially available. For example, when R^2 is H or methyl, the compounds of Formula V are PEG or mPEG compounds, respectively, which are commercially available from Aldrich of Milwaukee, Wisconsin; Fluka of Switzerland, and/or TCI America of Portland, Oregon.

30 When R^2 is a lipophilic moiety such as, for example, higher alkyl, fatty acid, an ester of a fatty acid, cholestryl, or adamantyl, the compounds of Formula V may be provided by

various methods as will be understood by those skilled in the art. The compounds of Formula V are preferably provided as follows:



R^2 is a lipophilic moiety, preferably higher alkyl, fatty acid ester, cholesteryl, or adamantly, more preferably a lower alkyl ester of a fatty acid, and most preferably an ethyl ester of a fatty acid having from 1 to 18 carbon atoms.

R^3 is H, benzyl, trityl, tetrahydropyran, or other alcohol protecting groups as will be understood by those skilled in the art.

X_2^+ is a positive ion as described above with respect to X^+ .

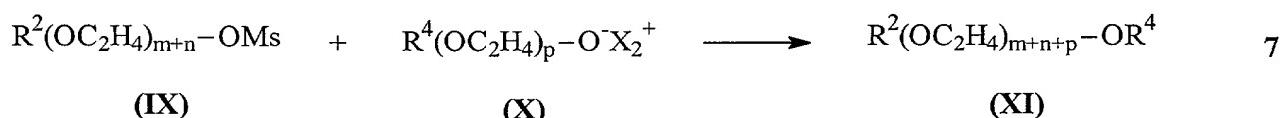
The value of m is as described above.

Regarding reaction 4, a mixture of compounds of Formula VI is reacted with a mixture of compounds of Formula VII under reaction conditions similar to those described above with reference to reaction 1. The mixture of compounds of Formula VI is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula VI have the same molecular weight. More preferably, the mixture of compounds of Formula VI is a monodispersed mixture. The mixture of compounds of Formula VII is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula VII have the same molecular weight. More preferably, the mixture of compounds of Formula VII is a monodispersed mixture.

Regarding reaction 5, the compound of Formula VIII may be hydrolyzed to convert the R³ moiety into an alcohol by various methods as will be understood by those skilled in the art. When R³ is benzyl or trityl, the hydrolysis is preferably performed utilizing H₂ in the presence of a palladium-charcoal catalyst as is known by those skilled in the art. Of course, when R³ is H, reaction 5 is unnecessary.

The compound of Formula VI may be commercially available or be provided as described above with reference to reaction 3. The compound of Formula VII may be provided as described above with reference to reaction 2.

Substantially monodispersed mixtures of polymers comprising PEG moieties and having the structure of Formula III above can further be reacted with other substantially monodispersed polymers comprising PEG moieties in order to extend the PEG chain. For example, the following scheme may be employed:



Ms, m and n are as described above with reference to reaction 1; p is similar to n and m, and X_2^+ is similar to X^+ as described above with reference to reaction 1. Q is as described above with reference to reaction 3. R^2 is as described above with reference to reaction 1 and is preferably lower alkyl. R^1 is H. Reaction 6 is preferably performed in a manner similar to that described above with reference to reaction 3. Reaction 7 is preferably performed in a manner similar to that described above with reference to reaction 1. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula III have the same molecular weight, and, more preferably, the mixture of compounds of Formula III is a monodispersed mixture. The mixture of compounds of Formula X is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula X have the same molecular weight, and, more preferably, the mixture of compounds of Formula X is a monodispersed mixture.

A process according to embodiments of the present invention is illustrated by the scheme shown in **Figure 1**, which will now be described. The synthesis of substantially monodispersed polyethylene glycol-containing oligomers begins by the preparation of the monobenzyl ether (**1**) of a substantially monodispersed polyethylene glycol. An excess of a commercially available substantially monodispersed polyethylene glycol is reacted with benzyl chloride in the presence of aqueous sodium hydroxide as described by Coudert *et al*

(*Synthetic Communications*, **16**(1): 19-26 (1986)). The sodium salt of **1** is then prepared by the addition of NaH, and this sodium salt is allowed to react with the mesylate synthesized from the ester of a hydroxyalkanoic acid (**2**). The product (**3**) of the displacement of the mesylate is debenzylated *via* catalytic hydrogenation to obtain the alcohol (**4**). The mesylate **5** (**5**) of this alcohol may be prepared by addition of methanesulfonyl chloride and used as the electrophile in the reaction with the sodium salt of the monomethyl ether of a substantially monodispersed polyethylene glycol derivative, thereby extending the polyethylene glycol portion of the oligomer to the desired length, obtaining the elongated ester (**6**). The ester may be hydrolyzed to the acid (**7**) in aqueous base and transformed into the activated ester (**8**) by **10** reaction with a carbodiimide and N-hydroxysuccinimide. While the oligomer illustrated in **15** **Figure 1** is activated using N-hydroxysuccinimide, it is to be understood that various other reagents may be used to activate oligomers of the present invention including, but not limited to, active phenyl chloroformates such as *para*-nitrophenyl chloroformate, phenyl chloroformate, 3,4-phenyldichloroformate, and 3,4-phenyldichloroformate; tresylation; and acetal formation.

Still referring to **Figure 1**, q is from 1 to 24. Preferably, q is from 1 to 18, and q is more preferably from 4 to 16. R⁴ is a moiety capable of undergoing hydrolysis to provide the carboxylic acid. R⁴ is preferably lower alkyl and is more preferably ethyl. The variables n and m are as described above with reference to reaction **1**.

20 All starting materials used in the procedures described herein are either commercially available or can be prepared by methods known in the art using commercially available starting materials.

The present invention will now be described with reference to the following examples. It should be appreciated that these examples are for the purposes of illustrating **25** aspects of the present invention, and do not limit the scope of the invention as defined by the claims.

EXAMPLES

Examples 1 through 10

30 Reactions in Examples 1 through 10 were carried out under nitrogen with magnetic stirring, unless otherwise specified. "Work-up" denotes extraction with an organic solvent, washing of the organic phase with saturated NaCl solution, drying (MgSO₄), and evaporation

(rotary evaporator). Thin layer chromatography was conducted with Merck glass plates precoated with silica gel 60°F - 254 and spots were visualized by iodine vapor. All mass spectra were determined by Macromolecular Resources Colorado State University, CO and are reported in the order m/z, (relative intensity). Elemental analyses and melting points were 5 performed by Galbraith Laboratories, Inc., Knoxville, TN. Examples 1-10 refer to the scheme illustrated in **Figure 2**.

Example 1

8-Methoxy-1-(methylsulfonyl)oxy-3,6-dioxaoctane (9)

10 A solution of non-polydispersed triethylene glycol monomethyl ether molecules (4.00 mL, 4.19 g, 25.5 mmol) and triethylamine (4.26 mL, 3.09 g, 30.6 mmol) in dry dichloromethane (50 mL) was chilled in an ice bath and place under a nitrogen atmosphere. A solution of methanesulfonyl chloride (2.37 mL, 3.51 g, 30.6 mmol) in dry dichloromethane (20 mL) was added dropwise from an addition funnel. Ten minutes after the completion of 15 the chloride addition, the reaction mixture was removed from the ice bath and allowed to come to room temperature. The mixture was stirred for an additional hour, at which time TLC (CHCl₃ with 15% MeOH as the elutant) showed no remaining triethylene glycol monomethyl ether.

20 The reaction mixture was diluted with another 75 mL of dichloromethane and washed successively with saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo to give a non-polydispersed mixture of compounds **9** as a clear oil (5.31g, 86%).

Example 2

Ethylene glycol mono methyl ether (10) (m=4,5,6)

25 To a stirred solution of non-polydispersed compound **11** (35.7 mmol) in dry DMF (25.7 mL), under N₂ was added in portion a 60% dispersion of NaH in mineral oil, and the mixture was stirred at room temperature for 1 hour. To this salt **12** was added a solution of non-polydispersed mesylate **9** (23.36) in dry DMF (4 ml) in a single portion, and the mixture 30 was stirred at room temperature for 3.5 hours. Progress of the reaction was monitored by TLC (12% CH₃OH-CHCl₃). The reaction mixture was diluted with an equal amount of 1N HCl, and extracted with ethyl acetate (2 x 20 ml) and discarded. Extraction of aqueous

solution and work-up gave non-polydispersed polymer **10** (82 -84% yield).

Example 3

3,6,9,12,15,18,21-Heptaoxadocosanol (10) (m=4)

5 Oil; Rf 0.46 (methanol : chloroform = 3:22); MS m/z calc'd for C₁₅H₃₂O₈ 340.21 (M⁺+1), found 341.2.

Example 4

3,6,9,12,15,18,21,24-Octaoxapentacosanol (10) (m=5)

10 Oil; Rf 0.43 (methanol : chloroform = 6:10); MS m/z calc'd for C₁₇H₃₆O₉ 384.24 (M⁺+1), found 385.3.

Example 5

3,6,9,12,15,18,21,24,27-Nonaoxaoctacosanol (10) (m=6)

15 Oil; Rf 0.42 (methanol : chloroform = 6:10); MS m/z calc'd for C₁₉H₄₀O₁₀ 428.26 (M⁺+1), found 429.3.

Example 6

20-methoxy-1-(methylsulfonyl)oxy-3,6,9,12,15,18-hexaoxaeicosane (14)

20 Non-polydispersed compound **14** was obtained in quantitative yield from the alcohol **13** (m=4) and methanesulfonyl chloride as described for **9**, as an oil; Rf 0.4 (ethyl acetate : acetonitrile = 1:5); MS m/z calc'd for C₁₇H₃₇O₁₀ 433.21 (M⁺+1), found 433.469.

Example 7

Ethylene glycol mono methyl ether (15) (m=3,4,5)

The non-polydispersed compounds **15** were prepared from a diol by using the procedure described above for compound **10**.

Example 8

3,6,9,12,15,18,21,24,27,30-Decaoxaheneicosanol (15) (m=3)

30 Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for C₂₁H₄₄O₁₁ 472.29 (M⁺+1), found 472.29.

Example 9

3,6,9,12,15,18,21,24,27,30,33-Unecaoxatetracosanol (15) (m=4)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for C₂₃H₄₈O₁₂ 516.31

5 (M⁺+1), found 516.31.

Example 10

3,6,9,12,15,18,21,24,27,30,33,36-Dodecaoxaheptatricosanol (15) (m=5)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for C₂₅H₅₂O₁₃ 560.67

10 (M⁺+1), found 560.67.

Examples 11 through 18 refer to the scheme illustrated in **Figure 3**.

Example 11

Hexaethylene glycol monobenzyl ether (16)

An aqueous sodium hydroxide solution prepared by dissolving 3.99 g (100 mmol) NaOH in 4 ml water was added slowly to non-polydispersed hexaethylene glycol (28.175 g, 25 ml, 100 mmol). Benzyl chloride (3.9 g, 30.8 mmol, 3.54 ml) was added and the reaction mixture was heated with stirring to 100°C for 18 hours. The reaction mixture was then cooled, diluted with brine (250 ml) and extracted with methylene chloride (200 ml x 2). The combined organic layers were washed with brine once, dried over Na₂SO₄, filtered and concentrated in vacuo to a dark brown oil. The crude product mixture was purified *via* flash chromatography (silica gel, gradient elution: ethyl acetate to 9/1 ethyl acetate/methanol) to yield 8.099 g (70 %) of non-polydispersed **16** as a yellow oil.

25

Example 12

Ethyl 6-methylsulfonyloxyhexanoate (17)

A solution of non-polydispersed ethyl 6-hydroxyhexanoate (50.76 ml, 50.41 g, 227 mmol) in dry dichloromethane (75 ml) was chilled in a ice bath and placed under a nitrogen atmosphere. Triethylamine (34.43 ml, 24.99 g, 247 mmol) was added. A solution of methanesulfonyl chloride (19.15 ml, 28.3 g, 247 mmol) in dry dichloromethane (75 ml) was added dropwise from an addition funnel. The mixture was stirred for three and one half

hours, slowly being allowed to come to room temperature as the ice bath melted. The mixture was filtered through silica gel, and the filtrate was washed successively with water, saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo to a pale yellow oil. Final purification of the crude product was
5 achieved by flash chromatography (silica gel, 1/1 hexanes/ethyl acetate) to give the non-polydispersed product (46.13 g, 85 %) as a clear, colorless oil. FAB MS: *m/e* 239 (M+H), 193 (M-C₂H₅O).

Example 13

10 **6-{2-[2-(2-{2-[2-(2-Benzylxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (18)**

Sodium hydride (3.225 g or a 60 % oil dispersion, 80.6 mmol) was suspended in 80 ml of anhydrous toluene, placed under a nitrogen atmosphere and cooled in an ice bath. A solution of the non-polydispersed alcohol **16** (27.3 g, 73.3 mmol) in 80 ml dry toluene was added to the NaH suspension. The mixture was stirred at 0°C for thirty minutes, allowed to come to room temperature and stirred for another five hours, during which time the mixture became a clear brown solution. The non-polydispersed mesylate **17** (19.21 g, 80.6 mmol) in 80 ml dry toluene was added to the NaH/alcohol mixture, and the combined solutions were stirred at room temperature for three days. The reaction mixture was quenched with 50 ml methanol and filtered through basic alumina. The filtrate was concentrated in vacuo and purified by flash chromatography (silica gel, gradient elution: 3/1 ethyl acetate/hexanes to ethyl acetate) to yield the non-polydispersed product as a pale yellow oil (16.52 g, 44 %).
15
20 FAB MS: *m/e* 515 (M+H).

Example 14

25 **6-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (19)**

Non-polydispersed benzyl ether **18** (1.03 g, 2.0 mmol) was dissolved in 25 ml ethanol. To this solution was added 270 mg 10 % Pd/C, and the mixture was placed under a
30 hydrogen atmosphere and stirred for four hours, at which time TLC showed the complete disappearance of the starting material. The reaction mixture was filtered through Celite 545 to remove the catalyst, and the filtrate was concentrated in vacuo to yield the non-

polydispersed title compound as a clear oil (0.67 g, 79 %). FAB MS: *m/e* 425 (M+H), 447 (M+Na).

Example 15

5 **6-{2-[2-(2-{2-[2-(2-methylsulfonylethoxy)ethoxy]ethoxy}-ethoxy]-ethoxy}-hexanoic acid ethyl ester (20)**

The non-polydispersed alcohol **19** (0.835 g, 1.97 mmol) was dissolved in 3.5 ml dry dichloromethane and placed under a nitrogen atmosphere. Triethylamine (0.301 ml, 0.219 g, 2.16 mmol) was added and the mixture was chilled in an ice bath. After two minutes, the 10 methanesulfonyl chloride (0.16 ml, 0.248 g, 2.16 mmol) was added. The mixture was stirred for 15 minutes at 0°C, then at room temperature for two hours. The reaction mixture was filtered through silica gel to remove the triethylammonium chloride, and the filtrate was washed successively with water, saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column 15 chromatography (silica gel, 9/1 ethyl acetate/methanol) to give non-polydispersed compound **20** as a clear oil (0.819 g, 83 %). FAB MS: *m/e* 503 (M+H).

Example 16

20 **6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy]-ethoxy}-hexanoic acid ethyl ester (21)**

NaH (88 mg of a 60 % dispersion in oil, 2.2 mmol) was suspended in anhydrous toluene (3 ml) under N₂ and chilled to 0 °C. Non-polydispersed diethylene glycol monomethyl ether (0.26 ml, 0.26 g, 2.2 mmol) that had been dried *via* azeotropic distillation with toluene was added. The reaction mixture was allowed to warm to room temperature and 25 stirred for four hours, during which time the cloudy grey suspension became clear and yellow and then turned brown. Mesylate **20** (0.50 g, 1.0 mmol) in 2.5 ml dry toluene was added. After stirring at room temperature over night, the reaction was quenched by the addition of 2 ml of methanol and the resultant solution was filtered through silica gel. The filtrate was concentrated in vacuo and the FAB MS: *m/e* 499 (M+H), 521 (M+Na). Additional 30 purification by preparatory chromatography (silica gel, 19/3 chloroform/methanol) provided the non-polydispersed product as a clear yellow oil (0.302 g 57 %). FAB MS: *m/e* 527 (M+H), 549 (M+Na).

Example 17

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid (22)

5 Non-polydispersed ester **21** (0.25 g, 0.46 mmol) was stirred for 18 hours in 0.71 ml of 1 N NaOH. After 18 hours, the mixture was concentrated in vacuo to remove the alcohol and the residue dissolved in a further 10 ml of water. The aqueous solution was acidified to pH 2 with 2 N HCl and the product was extracted into dichloromethane (30 ml x 2). The combined organics were then washed with brine (25 ml x 2), dried over Na₂SO₄, filtered and
10 concentrated in vacuo to yield the non-polydispersed title compound as a yellow oil (0.147 g, 62 %). FAB MS: *m/e* 499 (M+H), 521 (M+Na).

Example 18

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy}-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (23)

15 Non-polydispersed acid **22** (0.209 g, 0.42 mmol) were dissolved in 4 ml of dry dichloromethane and added to a dry flask already containing NHS (N-hydroxysuccinimide) (57.8 mg, 0.502 mmol) and EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (98.0 mg, 0.502 mmol) under a N₂ atmosphere. The solution was stirred at room temperature overnight and filtered through silica gel to remove excess reagents and the urea formed from the EDC. The filtrate was concentrated in vacuo to provide the non-polydispersed product as a dark yellow oil (0.235 g, 94 %). FAB MS: *m/e* 596 (M+H), 618 (M+Na).

20 Examples 19 through 24 refer to the scheme illustrated in **Figure 4**.

Example 19

Mesylate of triethylene glycol monomethyl ether (24)

To a solution of CH₂Cl₂ (100 mL) cooled to 0°C in an ice bath was added non-polydispersed triethylene glycol monomethyl ether (25 g, 0.15 mol). Then triethylamine (29.5 mL, 0.22 mol) was added and the solution was stirred for 15 min at 0°C, which was followed by dropwise addition of methanesulfonyl chloride (13.8 mL, 0.18 mol, dissolved in

20 mL CH₂Cl₂). The reaction mixture was stirred for 30 min at 0°C, allowed to warm to room temperature, and then stirred for 2 h. The crude reaction mixture was filtered through Celite (washed CH₂Cl₂ ~200 mL), then washed with H₂O (300 mL), 5% NaHCO₃ (300 mL), H₂O (300 mL), sat. NaCl (300 mL), dried MgSO₄, and evaporated to dryness. The oil was 5 then placed on a vacuum line for ~2h to ensure dryness and afforded the non-polydispersed title compound as a yellow oil (29.15 g, 80% yield).

Example 20

Heptaethylene glycol monomethyl ether (25)

To a solution of non-polydispersed tetraethylene glycol (51.5 g, 0.27 mol) in THF (1L) was added potassium t-butoxide (14.8 g, 0.13 mol, small portions over ~30 min). The reaction mixture was then stirred for 1h and then **24** (29.15 g, 0.12 mol) dissolved in THF (90 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH₂Cl₂, ~200 mL) and evaporated to dryness. 10 The oil was then dissolved in HCl (250 mL, 1 N) and washed with ethyl acetate (250 mL) to remove excess **24**. Additional washings of ethyl acetate (125 mL) may be required to remove remaining **24**. The aqueous phase was washed repetitively with CH₂Cl₂ (125 mL volumes) until most of the **25** has been removed from the aqueous phase. The first extraction will 15 contain **24**, **25**, and dicoupled side product and should be back extracted with HCl (125 mL, 1N). The organic layers were combined and evaporated to dryness. The resultant oil was 20 then dissolved in CH₂Cl₂ (100 mL) and washed repetitively with H₂O (50 mL volumes) until **25** was removed. The aqueous fractions were combined, total volume 500 mL, and NaCl was added until the solution became cloudy and then was washed with CH₂Cl₂ (2 x 500 mL). The 25 organic layers were combined, dried MgSO₄, and evaporated to dryness to afford a the non-polydispersed title compound as an oil (16.9 g, 41% yield). It may be desirable to repeat one or more steps of the purification procedure to ensure high purity.

Example 21

8-Bromoocanoate (26)

To a solution of 8-bromoocanoic acid (5.0 g, 22 mmol) in ethanol (100 mL) was 30 added H₂SO₄ (0.36 mL, 7.5 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H₂O (100 mL), sat.

NaHCO₃ (2 x 100 mL), H₂O (100 mL), dried MgSO₄, and evaporated to dryness to afford a clear oil (5.5 g, 98% yield).

Example 22

5 **Synthesis of MPEG7-C8 ester (27)**

To a solution of the non-polydispersed compound **25** (3.0 g, 8.8 mmol) in ether (90 mL) was added potassium t-butoxide (1.2 g, 9.6 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of the non-polydispersed compound **26** (2.4 g, 9.6 mmol), dissolved in ether (10 mL), was added and the reaction mixture was stirred overnight. The 10 crude reaction mixture was filtered through Celite (washed CH₂Cl₂, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H₂O (2 x 200 mL), dried MgSO₄, and evaporated to dryness. Column chromatography (Silica, ethyl acetate to ethyl acetate/methanol, 10:1) was performed and afforded the non-polydispersed title compound as a clear oil (0.843 g, 19% yield).

15

Example 23

MPEG7-C8 acid (28)

To the oil of the non-polydispersed compound **27** (0.70 g, 1.4 mmol) was added 1N NaOH (2.0 mL) and the reaction mixture was stirred for 4h. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH₂Cl₂ (2 x 50 mL). The organic layers were combined, washed sat. NaCl, dried MgSO₄, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.35 g, 53% yield).

20

Example 24

25 **Activation of MPEG7-C8 acid (29)**

Non-polydispersed mPEG7-C8-acid **28** (0.31g, 0.64 mmol) was dissolved in 3 ml of anhydrous methylene chloride and then solution of N-hydroxysuccinimide (0.079g, 0.69 mmol) and EDCI·HCl (135.6 mg, 0.71 mmol) in anhydrous methylene chloride added. Reaction was stirred for several hours, then washed with 1N HCl, water, dried over MgSO₄, 30 filtered and concentrated. Crude material was purified by column chromatography, concentrated to afford the non-polydispersed title compound as a clear oil and dried *via* vacuum.

Examples 25 through 29 refer to the scheme illustrated in **Figure 5**.

Example 25

5 **10-hydroxydecanoate (30)**

To a solution of non-polydispersed 10-hydroxydecanoic acid (5.0 g, 26.5 mmol) in ethanol (100 mL) was added H₂S0₄ (0.43 mL, 8.8 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H₂O (100 mL), sat. NaHCO₃ (2 x 100 mL), H₂O (100 mL), dried MgSO₄, and 10 evaporated to dryness to afford the non-polydispersed title compound as a clear oil (6.9 g, 98% yield).

Example 26

15 **Mesylate of 10-hydroxydecanoate (31)**

To a solution of CH₂Cl₂ (27 mL) was added non-polydispersed 10-hydroxydecanoate 30 (5.6 g, 26 mmol) and cooled to 0°C in an ice bath. Then triethylamine (5 mL, 37 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (2.7 mL, 24 mmol) dissolved in CH₂Cl₂ (3 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH₂C1₂, 80 mL) and the filtrate was washed H₂O (100 mL), 5% NaHCO₃ (2 x 100 mL), H₂O (100 mL), sat. NaCl (100 mL), dried MgSO₄, and evaporated to dryness to afford the non-polydispersed title compound as a yellowish oil (7.42 g, 97% yield).

25 **Example 27**

MPEG₇-C₁₀ Ester (32)

To a solution of non-polydispersed heptaethylene glycol monomethyl ether 25 (2.5 g, 7.3 mmol) in tetrahydrofuran (100 mL) was added sodium hydride (0.194 g, 8.1 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of mesylate of non-polydispersed 10-hydroxydecanoate 31 (2.4 g, 8.1 mmol), dissolved in tetrahydrofuran (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH₂C1₂, ~200 mL) and evaporated to dryness. The

resultant oil was dissolved in ethyl acetate and washed H₂O (2 x 200 mL), dried MgSO₄, evaporated to dryness, chromatographed (silica, ethyl acetate/methanol, 10:1), and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a clear oil (0.570 g, 15% yield).

5

Example 28

MPEG₇-C₁₀ Acid (33)

To the oil of non-polydispersed mPEG₇-C₁₀ ester **32** (0.570 g, 1.1 mmol) was added 1N NaOH (1.6 mL) and the reaction mixture was stirred overnight. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH₂Cl₂ (2 x 50 mL). The organic layers were combined, washed sat. NaCl (2 x 50 mL), dried MgSO₄, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.340 g, 62% yield).

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Example 29

Activation of MPEG₇-C₁₀ Acid (34)

The non-polydispersed acid **33** was activated using procedures similar to those described above in Example 24.

20 Examples 30 and 31 refer to the scheme illustrated in **Figure 6**.

Example 30

Synthesis of C18(PEG6) Oligomer (36)

Non-polydispersed stearoyl chloride **35** (0.7g, 2.31 mmol) was added slowly to a mixture of PEG6 (5 g, 17.7 mmol) and pyridine (0.97g, 12.4 mmol) in benzene. The reaction mixture was stirred for several hours (~5). The reaction was followed by TLC using ethylacetate/methanol as a developing solvent. Then the reaction mixture was washed with water, dried over MgSO₄, concentrated and dried *via* vacuum. Purified non-polydispersed compound **36** was analyzed by FABMS: m/e 549/ M⁺H.

30

Example 31

Activation of C18(PEG6) Oligomer

Activation of non-polydispersed C18(PEG6) oligomer was accomplished in two steps:

- 5 1) Non-polydispersed stearoyl-PEG6 **36** (0.8 g, 1.46 mmol) was dissolved in toluene and added to a phosgene solution (10 ml, 20 % in toluene) which was cooled with an ice bath. The reaction mixture was stirred for 1 h at 0°C and then for 3 h at room temperature. Then phosgene and toluene were distilled off and the remaining non-polydispersed stearoyl PEG6 chloroformate **37** was dried over P₂O₅ overnight.
- 10 2) To a solution of non-polydispersed stearoyl PEG6 chloroformate **36** (0.78g, 1.27 mmol) and TEA (128 mg, 1.27 mmol) in anhydrous methylene chloride, N-hydroxy succinimide (NHS) solution in methylene chloride was added. The reaction mixture was stirred for 16 hours, then washed with water, dried over MgSO₄, filtered, concentrated and dried *via* vacuum to provide the non-polydispersed activated C18(PEG6) oligomer **38**.

15 Examples 32 through 37 refer to the scheme illustrated in **Figure 7**.

Example 32

Tetraethylene glycol monobenzylether (39)

- 20 To the oil of non-polydispersed tetraethylene glycol (19.4 g, 0.10 mol) was added a solution of NaOH (4.0 g in 4.0 mL) and the reaction was stirred for 15 mm. Then benzyl chloride (3.54 mL, 30.8 mmol) was added and the reaction mixture was heated to 100°C and stirred overnight. The reaction mixture was cooled to room temperature, diluted with sat. NaCl (250 mL), and washed CH₂Cl₂ (2 x 200 mL). The organic layers were combined, washed sat. NaCl, dried MgSO₄, and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a yellow oil (6.21 g, 71% yield).
- 25

Example 33

Mesylate of tetraethylene glycol monobenzylether (40)

- 30 To a solution of CH₂Cl₂ (20 mL) was added non-polydispersed tetraethylene glycol monobenzylether **39** (6.21 g, 22 mmol) and cooled to 0°C in an ice bath. Then triethylamine (3.2 mL, 24 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then

methanesulfonyl chloride (1.7 mL, 24 mmol) dissolved in CH₂Cl₂ (2 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH₂Cl₂, 80 mL) and the filtrate was washed H₂O (100 mL), 5% NaHCO₃ (2 x 100 mL), H₂O (100 mL), sat. NaCl (100 mL), and dried MgSO₄. The resulting yellow oil was chromatographed on a pad of silica containing activated carbon (10 g) to afford the non-polydispersed title compound as a clear oil (7.10 g, 89% yield).

Example 34

10 **Octaethylene glycol monobenzylether (41)**

To a solution of tetrahydrofuran (140 mL) containing sodium hydride (0.43 g, 18 mmol) was added dropwise a solution of non-polydispersed tetraethylene glycol (3.5 g, 18 mmol) in tetrahydrofuran (10 mL) and the reaction mixture was stirred for 1 h. Then mesylate of non-polydispersed tetraethylene glycol monobenzylether **40** (6.0 g, 16.5 mmol) dissolved in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed, CH₂Cl₂, 250 mL) and the filtrate was washed H₂O, dried MgSO₄, and evaporated to dryness. The resultant oil was chromatographed (silica, ethyl acetate/methanol, 10:1) and chromatographed (silica, chloroform/methanol, 25:1) to afford the non-polydispersed title compound as a clear oil (2.62 g, 34% yield).

Example 35

15 **Synthesis of Stearate PEG8-Benzyl (43)**

To a stirred cooled solution of non-polydispersed octaethylene glycol monobenzylether **41** (0.998 g, 2.07 mmol) and pyridine (163.9 mg, 2.07 mmol) was added non-polydispersed stearoyl chloride **42** (627.7 mg, 2.07 mmol) in benzene. The reaction mixture was stirred overnight (18 hours). The next day the reaction mixture was washed with water, dried over MgSO₄, concentrated and dried *via* vacuum. Then the crude product was chromatographed on flash silica gel column, using 10% methanol/90% chloroform. The fractions containing the product were combined, concentrated and dried *via* vacuum to afford the non-polydispersed title compound.

10

Example 36

Hydrogenolysis of Stearate-PEG8-Benzyl

To a methanol solution of non-polydispersed stearate-PEG8-Bzl **43** (0.854g 1.138 mmol) Pd/C(10%) (palladium, 10% wt. on activated carbon) was added. The reaction mixture was stirred overnight (18 hours) under hydrogen. Then the solution was filtered, concentrated and purified by flash column chromatography using 10% methanol/90% chloroform, fractions with R_f =0.6 collected, concentrated and dried to provide the non-polydispersed acid **44**.

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Example 37

Activation of C18(PEG8) Oligomer

Two step activation of non-polydispersed stearate-PEG8 oligomer was performed as described for stearate-PEG6 in Example 31 above to provide the non-polydispersed activated C18(PEG8) oligomer **45**.

25

Example 38

Synthesis of Activated Triethylene Glycol Monomethyl Oligomers

The following description refers to the scheme illustrated in **Figure 8**. A solution of toluene containing 20% phosgene (100 ml, approximately 18.7 g, 189 mmol phosgene) was chilled to 0°C under a N₂ atmosphere. Non-polydispersed mTEG (triethylene glycol, monomethyl ether, 7.8 g, 47.5 mmol) was dissolved in 25 mL anhydrous ethyl acetate and added to the chilled phosgene solution. The mixture was stirred for one hour at 0°C, then allowed to warm to room temperature and stirred for another two and one half hours. The remaining phosgene, ethyl acetate and toluene were removed via vacuum distillation to leave the non-polydispersed mTEG chloroformate **46** as a clear oily residue.

30

The non-polydispersed residue **46** was dissolved in 50 mL of dry dichloromethane to which was added TEA (triethylamine, 6.62 mL, 47.5 mmol) and NHS (N-hydroxysuccinimide, 5.8 g, 50.4 mmol). The mixture was stirred at room temperature under a dry atmosphere for twenty hours during which time a large amount of white precipitate appeared. The mixture was filtered to remove this precipitate and concentrated *in vacuo*. The resultant oil **47** was taken up in dichloromethane and washed twice with cold deionized water, twice with 1N HCl and once with brine. The organics were dried over

MgSO₄, filtered and concentrated to provide the non-polydispersed title compound as a clear, light yellow oil. If necessary, the NHS ester could be further purified by flash chromatography on silica gel using EtOAc as the elutant.

5

Example 39

Synthesis of Activated Palmitate-TEG Oligomers

The following description refers to the scheme illustrated in **Figure 9**. Non-polydispersed palmitic anhydride (5 g; 10 mmol) was dissolved in dry THF (20 mL) and stirred at room temperature. To the stirring solution, 3 mol excess of pyridine was added followed by non-polydispersed triethylene glycol (1.4 mL). The reaction mixture was stirred for 1 hour (progress of the reaction was monitored by TLC; ethyl acetate-chloroform; 3:7). At the end of the reaction, THF was removed and the product was mixed with 10% H₂SO₄ acid and extracted ethyl acetate (3 x 30 mL). The combined extract was washed sequentially with water, brine, dried over MgSO₄, and evaporated to give non-polydispersed product **48**. A solution of N,N'-disuccinimidyl carbonate (3 mmol) in DMF (~10 mL) is added to a solution of the non-polydispersed product **48** (1 mmol) in 10 mL of anhydrous DMF while stirring. Sodium hydride (3 mmol) is added slowly to the reaction mixture. The reaction mixture is stirred for several hours (e.g., 5 hours). Diethyl ether is added to precipitate the activated oligomer. This process is repeated 3 times and the product is finally dried.

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Example 40

Synthesis of Activated Hexaethylene Glycol Monomethyl Oligomers

The following description refers to the scheme illustrated in **Figure 10**. Non-polydispersed activated hexaethylene glycol monomethyl ether was prepared analogously to that of non-polydispersed triethylene glycol in Example 39 above. A 20% phosgene in toluene solution (35 mL, 6.66 g, 67.4 mmol phosgene) was chilled under a N₂ atmosphere in an ice/salt water bath. Non-polydispersed hexaethylene glycol **50** (1.85 mL, 2.0 g, 6.74 mmol) was dissolved in 5 mL anhydrous EtOAc and added to the phosgene solution *via* syringe. The reaction mixture was kept stirring in the ice bath for one hour, removed and stirred a further 2.5 hours at room temperature. The phosgene, EtOAc, and toluene were removed by vacuum distillation, leaving non-polydispersed compound **51** as a clear, oily residue.

The non-polydispersed residue **51** was dissolved in 20 mL dry dichloromethane and placed under a dry, inert atmosphere. Triethylamine (0.94 mL, 0.68 g, 6.7 mmol) and then NHS (N-hydroxy succinimide, 0.82 g, 7.1 mmol) were added, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was filtered through silica gel to remove the white precipitate and concentrated *in vacuo*. The residue was taken up in dichloromethane and washed twice with cold water, twice with 1 N HCl and once with brine. The organics were dried over Na₂SO₄, filtered and concentrated. Final purification was done via flash chromatography (silica gel, EtOAc) to obtain the UV active non-polydispersed NHS ester **52**.

10

Example 41

Synthesis of Polypeptide-Oligomer Conjugates

Mixtures of polypeptide-oligomer conjugates according to the present invention are synthesized as follows. A mixture of the polypeptide is dissolved in anhydrous DMF. Then TEA and a mixture of an activated oligomer of Example 18, 24, 29, 31, 37, 38, 39 or 40 in anhydrous THF is added. The reaction mixture is then stirred, preferably for 1 hour. The reaction mixture is acidified (for example, by adding 2 mL of 0.1% TFA in water). The reaction is followed by HPLC. The reaction mixture is concentrated and purified by preparative liquid chromatography (for example, using Waters PrepLC™ 4000 RP Vydac C18 Protein and peptide, 1x25 column, water/acetonitrile with 0.1%TFA, detection at 280 nm). Peaks corresponding to mono- or multi-conjugated compounds are isolated. Samples may be analyzed by MALDI-MS.

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The procedure of Example 41 is performed and the polypeptide is an

adrenocorticotrophic hormone peptide.

30

The procedure of Example 41 is performed and the polypeptide is an adrenomedullin

peptide.

Example 43

Example 44

The procedure of Example 41 is performed and the polypeptide is an allatostatin peptide.

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Example 45

The procedure of Example 41 is performed and the polypeptide is an amylin peptide.

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Example 46

The procedure of Example 41 is performed and the polypeptide is an amyloid beta-protein fragment peptide.

Example 47

The procedure of Example 41 is performed and the polypeptide is an angiotensin peptide.

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Example 48

The procedure of Example 41 is performed and the polypeptide is an antibiotic peptide.

Example 49

The procedure of Example 41 is performed and the polypeptide is an antigenic polypeptide.

Example 50

25 The procedure of Example 41 is performed and the polypeptide is an anti-microbial peptide.

Example 51

30 The procedure of Example 41 is performed and the polypeptide is an apoptosis related peptide.

Example 52

The procedure of Example 41 is performed and the polypeptide is an atrial natriuretic peptide.

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Example 53

The procedure of Example 41 is performed and the polypeptide is a bag cell peptide.

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Example 54

The procedure of Example 41 is performed and the polypeptide is a bombesin peptide.

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Example 55

The procedure of Example 41 is performed and the polypeptide is a bone GLA peptide.

Example 56

The procedure of Example 41 is performed and the polypeptide is a bradykinin peptide.

Example 57

The procedure of Example 41 is performed and the polypeptide is a brain natriuretic peptide.

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Example 58

The procedure of Example 41 is performed and the polypeptide is a C-peptide.

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Example 59

The procedure of Example 41 is performed and the polypeptide is a C-type natriuretic peptide.

150 mg of salmon calcitonin (MW 3432, 0.043 mmol) was dissolved in 30 ml of anhydrous DMF. Then TEA (35 µL) and the activated oligomer of Example 24 (42 mg,

0.067 mmol) in anhydrous THF (2 mL) was added. The reaction was stirred for 1 hour, then acidified with 2 mL of 0.1% TFA in water. The reaction was followed by HPLC. Then the reaction mixture was concentrated and purified by preparative liquid chromatography (Waters PrepLC™ 4000 RC Vydac C18 Protein and peptide, 1x25 column, water/acetonitrile with 0.1% TFA, detection at 280 nm). Two peaks, corresponding to mono- and di-conjugate were isolated. Samples were analyzed by MALDI-MS. MS for PEG7-octyl-sCT, mono-conjugate: 3897. MS for PEG7-octyl-sCT, di-conjugate: 4361.

5 A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 29. MS for PEG7-decyl-sCT, mono-conjugate: 3926. MS for PEG7-
10 decyl-sCT, di-conjugate: 4420.

A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 31. MS for stearate-PEG6-sCT, mono-conjugate: 4006. MS for stearate-PEG6-sCT, di-conjugate: 4582.

15 A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 37. MS for stearate-PEG8-sCT, mono-conjugate: 4095.

A similar procedure is used to conjugate salmon calcitonin with the activated oligomer of Example 18, 38, 39 and 40.

Example 61

20 The procedure of Example 41 is performed and the polypeptide is a calcitonin gene related peptide.

Example 62

25 The procedure of Example 41 is performed and the polypeptide is a CART peptide.

Example 63

The procedure of Example 41 is performed and the polypeptide is a casomorphin peptide.

Example 64

30 The procedure of Example 41 is performed and the polypeptide is a chemotactic peptide.

Example 65

The procedure of Example 41 is performed and the polypeptide is a cholecystokinin peptide.

5

Example 66

The procedure of Example 41 is performed and the polypeptide is a corticotropin releasing factor peptide.

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Example 67

The procedure of Example 41 is performed and the polypeptide is a cortistatin peptide.

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Example 68

The procedure of Example 41 is performed and the polypeptide is a dermorphin peptide.

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Example 69

The procedure of Example 41 is performed and the polypeptide is a dynorphin peptide.

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Example 70

The procedure of Example 41 is performed and the polypeptide is an endorphin peptide.

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Example 71

The procedure of Example 41 is performed and the polypeptide is an endothelin peptide.

Example 72

The procedure of Example 41 is performed and the polypeptide is an ET_a receptor antagonist peptide.

5

Example 73

The procedure of Example 41 is performed and the polypeptide is an ET_b receptor antagonist peptide.

10

Example 74

The procedure of Example 41 is performed and the polypeptide is an enkephalin peptide.

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Example 75

The procedure of Example 41 is performed and the polypeptide is a fibronectin peptide.

Example 76

The procedure of Example 41 is performed and the polypeptide is a galanin peptide.

Example 77

The procedure of Example 41 is performed and the polypeptide is a gastrin peptide.

Example 78

The procedure of Example 41 is performed and the polypeptide is a glucagon peptide.

Example 79

The procedure of Example 41 is performed and the polypeptide is a Gn-RH associated

25 peptide.

Example 80

The procedure of Example 41 is performed and the polypeptide is a growth factor peptide.

30

Example 81

Human growth hormone was conjugated with the activated oligomers of Example 40

as illustrated in **Figure 10**. Human growth hormone (somatropin (rDNA origin) for injection), available under the trade name Saizen™ from Serono of Randolph, Massachusetts, was dissolved in DMSO such that the hGH was at a 0.58 mmol concentration. TEA (278 equivalents) was added and the solution was stirred for approximately ten minutes. Two 5 equivalents, five equivalents or thirty equivalents of activated hexaethylene glycol **52** was added from a 0.2 M solution of the activated oligomer in dry THF. Reactions were stirred at room temperature for 45 minutes to one hour. Aliquots of each reaction mixture were quenched in 600 µL of 0.1% TFA in water. HPLC comparison of the 2 polymer equivalent and the 5 polymer equivalent reaction mixtures vs. unconjugated hGH is shown in **Figure 14**.

10 HPLC analysis of the thirty polymer equivalent reaction is shown in **Figure 15**.

Samples of the conjugates for mass spectroscopy were purified *via* analytical HPLC using a reversed-phase C₁₈ column and a water/acetonitrile gradient. The entire peak from the 2 equivalent reaction mixture was collected, concentrated and analyzed using MALDI mass spectroscopy. The mass spectra of this material showed evidence of the presence of mono-conjugated, di-conjugated, tri-conjugated and tetra-conjugated hGH as well as some remaining unreacted hGH (**Figure 16**). The five equivalent reaction mixture was purified crudely according to polarity as indicated in **Figure 17**. MALDI mass spectra of the concentrated fractions (**Figure 18**, **Figure 19** and **Figure 20**) indicated that the level of conjugation of the protein increased with retention time. Electrospray mass spectra of fraction E, **Figure 21**, gave results consistent with the presence of hexa-conjugated hGH. The entire peak from the thirty polymer equivalent reaction mixture was collected and concentrated. Electrospray mass spectral analysis, **Figure 22**, showed deca- and higher conjugated material.

A similar procedure is used to conjugate hGH with the activated oligomer of Example 25 18, 24, 29, 31 or 37.

Example 82

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated Palmitate-TEG Oligomers

30 Procedures similar to those described above in Example 81 were performed using the activated polymer from Example 39. Progress of conjugation was checked by HPLC by taking 20 µL of the conjugated reaction mixture in a vial and diluting with 100 µL of 0.1% TFA-water-IPA (1:1), the results of which are illustrated in **Figure 23**. After 2 hours, the

reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC.

Example 83

5 **Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated TEG Oligomers**

One equivalent of human growth hormone (hGH) (somatropin (rDNA origin) for injection), available under the trade name SaizenTM from Serono of Randolph, Massachusetts was dissolved in DMSO (1 mg/125 µL) and stirred at room temperature for 2-4 minutes.

10 Two equivalents TEA was added followed by two equivalents of the activated oligomer of Example 38, which was dissolved in THF. After 2 hours, the reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC as illustrated in **Figure 24**.

A similar procedure five equivalents TEA and five equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in **Figure 25**. The mobile phase and elution time were as follows:

Time	mL/min	Solvent A	Solvent B
0	3.5	80	20
55	3.5	0	100

The pooled fraction was lyophilized into a white powder. The mass spectra for the compound are illustrated in **Figures 26 and 27**.

20 A similar procedure utilizing nine equivalents TEA and nine equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in **Figure 28**.

Example 84

25 The procedure of Example 41 is performed and the polypeptide is a GTP-binding peptide.

Example 85

The procedure of Example 41 is performed and the polypeptide is a guanylin peptide.

Example 86

The procedure of Example 41 is performed and the polypeptide is an inhibin peptide.

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Example 87

Synthesis of Insulin-Oligomer Conjugates

To human insulin (zinc or zinc free, 2g, 0.344 mmol based on dry weight) in 25 mL dimethylsulfoxide (> 99% purity) at 22±4°C was added 8 mL triethyl amine (> 99% purity). The resulting mixture was stirred for 5 to 10 minutes at 22±4°C. To the above was rapidly added the activated oligomer of Example 18 above (0.188 g, 0.36 mmol based on 100% activation) in 7.5 mL acetonitrile under stirring at 22±4°C. The solution was stirred for 45 minutes and quenched with acetic acid solution with maintaining the temperature below 27°C. The reaction was monitored by analytical HPLC. This reaction condition produces PEG7-hexyl-insulin, monoconjugated at the B29 position (PEG7-hexyl-insulin, B29 monoconjugated) at yield 40-60%. The crude reaction mixture (PEG7-hexyl-insulin, B29 monoconjugated, 40-60%, unreacted insulin 8-25%, related substances 15-35%) was dialyzed or difiltered (3000-3500 molecular weight cut off, MWCO) to remove organic solvents and small molecular weight impurities, exchanged against ammonium acetate buffer and lyophilized.

The conjugation reaction of PEG7-hexyl-insulin, monoconjugated at the B29 position, was monitored by analytical HPLC. This analytical HPLC method used a Waters Delta-Pak C18 column, 150 x 3.9 mm I.D., 5µm, 300 Å. The solvent system consisted of Solvent B: 0.1% TFA in 50/50 methanol/water, and Solvent D: 0.1% TFA in methanol. The gradient system was as follows:

Time (min)	% Solvent B	% Solvent D	Flow rate (mL/min)
Initial (0)	100	0	1.00
20	40	60	1.00
25	100	0	1.00

25

A similar procedure is used to provide non-polydispersed mixtures of insulin-oligomer conjugates using the activated oligomers of Example 24, 29, 31, 37, 38, 39 and 40.

Example 88

The procedure of Example 41 is performed and the polypeptide is an interleukin peptide.

5

Example 89

The procedure of Example 41 is performed and the polypeptide is a leptin peptide.

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The procedure of Example 41 is performed and the polypeptide is a leucokinin peptide.

Example 91

The procedure of Example 41 is performed and the polypeptide is a luteinizing hormone-releasing hormone.

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Example 92

The procedure of Example 41 is performed and the polypeptide is a mastoparan peptide.

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Example 93

The procedure of Example 41 is performed and the polypeptide is a mast cell degranulating peptide.

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Example 94

The procedure of Example 41 is performed and the polypeptide is a melanocyte stimulating hormone peptide.

Example 95

The procedure of Example 41 is performed and the polypeptide is a morphiceptin peptide.

Example 96

The procedure of Example 41 is performed and the polypeptide is a motilin peptide.

Example 97

5 The procedure of Example 41 is performed and the polypeptide is a neuro-peptide.

Example 98

The procedure of Example 41 is performed and the polypeptide is a neuropeptide Y peptide.

10

Example 99

The procedure of Example 41 is performed and the polypeptide is a neurotropic factor peptide.

DRAFT
10
15
20
25

Example 100

The procedure of Example 41 is performed and the polypeptide is an orexin peptide.

Example 101

The procedure of Example 41 is performed and the polypeptide is an opioid peptide.

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Example 102

The procedure of Example 41 is performed and the polypeptide is an oxytocin peptide.

25

Example 103

The procedure of Example 41 is performed and the polypeptide is a PACAP peptide.

30

Example 104

The procedure of Example 41 is performed and the polypeptide is a pacreastatin peptide.

Example 105

The procedure of Example 41 is performed and the polypeptide is a pancreatic polypeptide.

5

Example 106

The procedure of Example 41 is performed and the polypeptide is a parathyroid hormone peptide.

Example 107

10 The procedure of Example 41 is performed and the polypeptide is a parathyroid hormone-related peptide.

Example 108

The procedure of Example 41 is performed and the polypeptide is a peptide T peptide.

Example 109

15 The procedure of Example 41 is performed and the polypeptide is a prolactin-releasing peptide.

Example 110

20 The procedure of Example 41 is performed and the polypeptide is a peptide YY peptide.

Example 111

25 The procedure of Example 41 is performed and the polypeptide is a renin substrate peptide.

Example 112

The procedure of Example 41 is performed and the polypeptide is a secretin peptide.

30

Example 113

The procedure of Example 41 is performed and the polypeptide is a somatostatin

peptide.

Example 114

The procedure of Example 41 is performed and the polypeptide is a substance P
5 peptide.

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Example 115

The procedure of Example 41 is performed and the polypeptide is a tachykinin
peptide.

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Example 116

The procedure of Example 41 is performed and the polypeptide is a thyrotropin-
releasing hormone peptide.

Example 117

The procedure of Example 41 is performed and the polypeptide is a toxin peptide.

Example 118

The procedure of Example 41 is performed and the polypeptide is a vasoactive
intestinal peptide.

Example 119

The procedure of Example 41 is performed and the polypeptide is a vasopressin
peptide.

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Example 120

The procedure of Example 41 is performed and the polypeptide is a virus related
peptide.

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Example 121

**Purification of B29 Modified PEG7-Hexyl-Insulin,
Monoconjugate, from the Crude Mixture**

PEG7-hexyl-insulin, B29 monoconjugated, was purified from the crude mixture of

Example 87 using a preparative HPLC system. Lyophilized crude mixture (0.5 g, composition: PEG7-hexyl-insulin, B29 monoconjugated, 40-60%, unreacted insulin 8-25%, related substances 15-35%) was dissolved in 5-10 mL 0.01 M ammonium acetate buffer, pH 7.4 and loaded to a C-18 reverse phase HPLC column (150 x 3.9 mm) equilibrated with 0.5% triethylamine/0.5% phosphoric acid buffer TEAP A). The column was eluted with a gradient flow using TEAP A and TEAP B (80% acetonitrile and 20% TEAP A) solvent system. The gradient system for preparative HPLC purification of PEG7-hexyl-insulin, B29 monoconjugate, from the crude mixture was as follows:

Time (min)	% TEAP A	% TEAP B	Flow rate (mL/min)
Initial (0)	70	30	30
45	64	36	30
105	60	40	30
115	40	60	30
125	15	85	30
135	15	85	30

Fractions were analyzed by HPLC and the product fractions that were > 97% purity of PEG7-hexyl-insulin, B29 monoconjugate, were pooled. The elution buffer and solvent were removed by dialysis or diafiltration (MWCO 3000-3500) against ammonium acetate buffer (0.01 M, pH 7.4) and exchanged into ammonium acetate buffer and lyophilized to produce white powder of PEG7-hexyl-insulin, B29 monoconjugate (purity >97%).

An analytical HPLC method using the same column and solvent system as the method used in Example 87 to monitor the reaction was used for analysis of PEG7-hexyl-insulin, B29 monoconjugate. However, the gradient conditions were as follows:

Time (min)	% Solvent B	% Solvent D	Flow rate (mL/min)
Initial (0)	100	0	1.00
30	10	90	1.00
35	100	0	1.00

Example 122

Determination of the Dispersity Coefficient for a Mixture of Human Insulin-Oligomer Conjugates

The dispersity coefficient of a mixture of human insulin-oligomer conjugates is

5 determined as follows. A mixture of human insulin-oligomer conjugates is provided, for example as described above in Example 87. A first sample of the mixture is purified via HPLC to separate and isolate the various human insulin-oligomer conjugates in the sample.

Assuming that each isolated fraction contains a purely monodispersed mixture of conjugates, "n" is equal to the number of fractions collected. The mixture may include one or more of the

10 following conjugates, which are described by stating the conjugation position followed by the degree of conjugation: Gly^{A1} monoconjugate; Phe^{B1} monoconjugate; Lys^{B29} monoconjugate; Gly^{A1}, Phe^{B1} diconjugate; Gly^{A1}, Lys^{B29} diconjugate; Phe^{B1}, Lys^{B29} diconjugate; and/or Gly^{A1}, Phe^{B1}, Lys^{B29} triconjugate. Each isolated fraction of the mixture is analyzed via mass spectroscopy to determine the mass of the fraction, which allows each isolated fraction to be categorized as a mono-, di-, or tri-conjugate and provides a value for the variable "M_i" for each conjugate in the sample.

A second sample of the mixture is analyzed via HPLC to provide an HPLC trace.

Assuming that the molar absorptivity does not change as a result of the conjugation, the weight percent of a particular conjugate in the mixture is provided by the area under the peak of the HPLC trace corresponding to the particular conjugate as a percentage of the total area under all peaks of the HPLC trace. The sample is collected and lyophilized to dryness to determine the anhydrous gram weight of the sample. The gram weight of the sample is multiplied by the weight percent of each component in the sample to determine the gram weight of each conjugate in the sample. The variable "N_i" is determined for a particular conjugate (the ith conjugate) by dividing the gram weight of the particular conjugate in the sample by the mass of the particular conjugate and multiplying the quotient by Avagadro's number (6.02205×10^{23} mole⁻¹), M_i, determined above, to give the number of molecules of the particular conjugate, N_i, in the sample. The dispersity coefficient is then calculated using n, M_i as determined for each conjugate, and N_i as determined for each conjugate.

Example 123

Cytosensor Studies for Insulin-Oligomer Conjugates

Colo 205 (colorectal adenocarcinoma cells from ATCC, catalog #CCL-222) cells that had been serum-deprived for approximately 18 hours were suspended in 3:1 Cytosensor low-buffer RPMI-1640 media: Cytosensor agarose entrapment media and seeded into Cytosensor capsule cups at 100,000 cells/10 µL droplet. Cells were allowed to equilibrate on the Cytosensor to the low-buffer RPMI-1640 media at a flow rate of 100 µL per minute for approximately 3 hours until baseline acidification rates were stable. Insulin drugs (insulin or insulin conjugates) were diluted to 50nM in low-buffer RPMI-1640 media and applied to the cells for 20 minutes at 100 µL/minute. Following the exposure, the drug solutions were withdrawn and the cells were again perfused under the continuous flow of low-buffer media alone. Data collection continued until acidification rates returned to baseline levels (approximately one hour from application of drug solutions). The results are illustrated in **Figure 29**. As used in **Figure 29**, insulin is human insulin; PEG4 is a non-polydispersed mixture of mPEG4-hexyl-insulin, B29 monoconjugates; PEG10 is a non-polydispersed mixture of mPEG10-hexyl-insulin, B29 monoconjugates; PEG7 is a non-polydispersed mixture of mPEG7-hexyl-insulin, B29 monoconjugates; PEG7_{AVG} is a polydispersed mixture of mPEG7_{AVG}-hexyl-insulin, B29 monoconjugates.

Example 124

Enzymatic Stability of Insulin-Oligomer Conjugates

Chymotrypsin digests were conducted in a phosphate buffer, pH 7.4, at 37°C in a shaking water bath. The insulin/insulin conjugate concentration was 0.3 mg/mL. The chymotrypsin concentration was 2Units/mL. 100 µL samples were removed at the indicated time points and quenched with 25 µL of a 1:1 mixture of 0.1% trifluoroacetic acid:isopropyl alcohol. Samples were analyzed by reverse phase HPLC and the relative concentrations of insulin/insulin conjugate were determined by calculating the areas under the curves.

As used in **Figure 30**, insulin is human insulin; PEG4 is a non-polydispersed mixture of mPEG4-hexyl-insulin, B29 monoconjugates; PEG10 is a non-polydispersed mixture of mPEG10-hexyl-insulin, B29 monoconjugates; PEG7 is a non-polydispersed mixture of

mPEG7-hexyl-insulin, B29 monoconjugates; PEG7_{Avg} is a polydispersed mixture of mPEG7_{Avg}-hexyl-insulin, B29 monoconjugates.

Example 125

5 **Dose Dependent Activity for Insulin-Oligomer Conjugates**

An effective animal model for evaluating formulations uses normal fasted beagle dogs. These dogs are given from 0.25 mg/kg to 1.0 mg/kg of insulin conjugates to evaluate the efficacy of various formulations. This model was used to demonstrate that insulin conjugates according to the present invention provide lower glucose levels in a dose dependent manner better than polydispersed insulin conjugates, which are not part of the present invention and are provided for comparison purposes.

The protocol for dog experiments calls for a blood glucose measurement at time zero just before a drug is administered. The formulation in solid oral dosage form is then inserted into the dog's mouth. Blood is drawn at 15, 30, 60 and 120 minutes and glucose levels are measured and graphed. The lower the glucose levels, the better the activity of the insulin conjugate. In **Figure 31**, the glucose lowering, and thus the activity, of the conjugates of the present invention is shown to be dose dependent. For comparison purposes, **Figure 32** shows that the glucose lowering of polydispersed insulin conjugates in a capsule formulation, which are not a part of the present invention, is less dose dependent than conjugates of the present invention.

Example 126

Activity and Inter-Subject Variability for Insulin-Oligomer Conjugates

An effective animal model for evaluating formulations uses normal fasted beagle dogs. These dogs are given 0.25 mg/kg of insulin conjugates to evaluate the efficacy of various formulations. This model was used to demonstrate that insulin conjugates according to the present invention provide lower inter-subject variability and better activity than polydispersed insulin conjugates, which are not part of the present invention but are provided for comparison purposes.

30 The protocol for dog experiments calls for a blood glucose measurement at time zero just before a drug is administered. The oral liquid dosage formulation is then squirted into the back of the dog's mouth. In each case, the dogs received 0.25 mg/kg of this solution.

Blood is drawn at 15, 30, 60 and 120 minutes and glucose levels are measured and graphed. The lower the glucose levels, the better the activity of the insulin conjugate. In **Figures 33, 34 and 35**, the results obtained with PEG4-hexyl-insulin, monoconjugate; PEG7-hexyl-insulin, monoconjugate; and PEG10-hexyl-insulin, monoconjugate, respectively, show these 5 PEG conjugates of the present invention result in less inter-subject variability and higher activity than the results shown in **Figure 36** for the polydispersed PEG7_{Avg}-hexyl-insulin, monoconjugate, which is not a part of the present invention and is provided for comparison purposes.

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Example 127

Cytosensor® Studies for Calcitonin-Oligomer Conjugates

T-47D cells (mammary ductal carcinoma cell line, obtained from American Type Culture Collection were suspended at a density of 1×10^7 cells/mL in running buffer (low-buffered, serum-free, bicarbonate-free RPMI 1640 medium from Molecular Devices of Sunnyvale, California. Approximately 100,000 cells were then immobilized in an agarose cell entrapment medium in a 10 μ L droplet and sandwiched between two 3- μ m polycarbonate membranes in a cytosensor capsule cup. Cytosensor capsule cups placed in sensor chambers on the Cytosensor® Microphysiometer were then held in very close proximity to pH-sensitive detectors. Running buffer was then pumped across the cells at a rate of 100 μ L/min except during 30-second intervals when the flow was stopped, and acidification of the running buffer in the sensor chamber was measured. Acidification rates were determined every 2 minutes. The temperature of the sensor chambers was 37°C. Cells were allowed to equilibrate in the sensor chambers for 2-3 hours prior to the start of the experiment during which time basal acidification rates were monitored. Cells were then exposed to test compounds (Salmon 15 Calcitonin or Octyl-Di-Calcitonin) diluted in running buffer at various nM concentration. Exposure of cells to test compounds occurred for the first 40 seconds of each 2 minute pump cycle in a repeating pattern for a total of 20 minutes. This allowed sufficient exposure of the cells to the test compounds to elicit a receptor-mediated response in cellular metabolism followed by approximately 50 seconds of flow of the running buffer containing no 20 compounds. This procedure rinsed away test solutions (which had a slightly lower pH than running buffer alone) from the sensor chamber before measuring the acidification rate. Thus, the acidification rates were solely a measure of cellular activity. A similar procedure was 30

used to obtain data for PEG7-octyl-sCT, monoconjugate (Octyl-Mono); PEG7-decyl-sCT, monoconjugate (Decyl-Mono); PEG7-decyl-sCT, diconjugate (Decyl-Di); stearate-PEG6-sCT, monoconjugate (PEG6 St. Mono); and stearate-PEG8-sCT, monoconjugate (PEG8 St. Mono). Data was analyzed for relative activity of compounds by calculating the Area Under
5 the Curve (AUC) for each cytosensor chamber acidification rate graph and plotted as a bar chart illustrated in **Figure 37** showing average AUC measurements taken from multiple experiments performed under the same experimental conditions.

Example 128

10 **Enzymatic Stability for Calcitonin-Oligomer Conjugates**

Compounds, supplied as lyophilized powders, are resuspended in 10 mM phosphate buffer pH 7.4 and then submitted for concentration determination by HPLC. The phosphate buffer is used to create a solution with a pH that is optimum for activity of each particular gut enzyme. Aliquots of the compound thus prepared are transferred to 1.7 mL microcentrifuge tubes and shaken in a 37°C water bath for 15 minutes to allow compounds to equilibrate to temperature. After 15 minutes, 2 µL of the appropriate concentrated gut enzyme is added to each tube to achieve the final concentration desired. Chymotrypsin and trypsin are resuspended in 1 mM HCl. Also, as a control, compounds are treated with 2 µL of 1 mM HCl. Immediately following additions, 100 µL of sample is removed from the control tube and quenched with either 25 µL of chymotrypsin/trypsin quenching solution (1:1 1%
15 TFA:Isopropanol). This sample will serve as T=0 min. A sampling procedure is repeated at various time intervals depending on the gut enzyme used. Chymotrypsin has 15, 30 and 60 minute samples. Trypsin has 30, 60, 120 and 180 minute samples. Once all points have been acquired, a final sample is removed from the control tube to make sure that observed
20 degradation is not temperature or buffer related. The chymotrypsin and trypsin samples may be collected directly into HPLC vials. RP-HPLC (acetonitrile gradient) is used to determine AUC for each sample and % degradation is calculated based from the T=0 min control. The results are provided below in Tables 1 to 4.

Table 1

% Remaining Following 0.5 U/mL Chymotrypsin Digest
of PEG7-Octyl-Salmon Calcitonin, Diconjugate

Time	Non-Formulated				Buffered Formulation		
	15	30	60	60	88	73	61
15	63	71	68	69	88	86	88
30	34	48	50	46	73	88	86
60	6	15	20	15	61	69	84
	Control				Control		
60	104	88	97	103	116	104	101

Table 2

% Remaining Following 0.5 U/mL Chymotrypsin Digest of Salmon Calcitonin
(for comparison purposes; not part of the invention)

Time	Non-Formulated					Buffered Formulation		
	10	15	30	60	60			
10	73	-	55	62	35	66	59	91
15	-	55	62	35	42	66	59	92
30	30	26	40	13	42	54	86	87
60	1.6	5	12	1	12	55	82	85
	Control					Control		
60	-	100	93	45	100	102	98	103

Table 3
 % Remaining following 1 U/mL Trypsin Digest
 of PEG7-Octyl-Salmon Calcitonin, Diconjugate

Time	Non-Formulated			
30	87	89	83	90
60	78	86	76	85
120	72	82	68	78
180	-	81	61	73
	Control			
60		103	100	
120	106	105	99	
180		104	99	

Table 4
 % Remaining following 1 U/mL Trypsin Digest of Salmon Calcitonin
 (for comparison purposes; not part of the invention)

Time	Non-Formulated			
30	80	50	82	87
60	66	28	69	76
120	44	7	46	59
180	-	2	31	46
	Control			
60		41	101	
120	69	16	102	
180		7	101	

Example 130

Activity and Inter-Subject Variability for Calcitonin-Oligomer Conjugates

Male CF-1 mice (Charles River, Raleigh, NC) weighing 20-25 g were housed in the Nobex vivarium in a light- (L:D cycle of 12:12, lights on at 0600 h), temperature- (21-23°C),
5 and humidity- (40-60 % relative humidity) controlled room. Animals were permitted free access to laboratory chow (PMI Nutrition) and tap water. Mice were allowed to acclimate to housing conditions for 48-72 hours prior to the day of experiment.

Prior to dosing, mice were fasted overnight and water was provided ad libitum. Mice
were randomly distributed into groups of five animals per time point and were administered a
10 single oral dose of a PEG7-octyl-sCT, diconjugate (Octyl Di) according to the present invention or salmon calcitonin (sCT or Calcitonin) for comparison purposes. Oral doses were administered using a gavaging needle (Popper #18, 5 cm from hub to bevel) at 10 mL/kg in the following 0.2 µg/mL phosphate-buffered PEG7-octyl-sCT, diconjugate, formulation:

Ingredient	Amount
PEG7-octyl-sCT, diconjugate	20 µg
Sodium-cholate	2.5 g
Sodium-deoxy-cholate	2.5 g
Sodium phosphate buffer, 100 mM, pH 7.4	q.s. to 100 g

The buffered formulation was prepared by adding 80 mL of phosphate buffer in a clean tared glass beaker. The sodium cholate was slowly added to the phosphate buffer with stirring until dissolved. The deoxy cholate was then added and stirring was continued until dissolved. The PEG7-octyl-sCT, diconjugate, solution equivalent to 20 µg was added. Finally, the remaining phosphate buffer was added to achieve a final weight of 100 g. Vehicle-control mice were used in all experiments. Dose-response curves were constructed using a single
20 time point 60 minutes after drug administration. These curves are illustrated in **Figures 38-41**.

At appropriate time points, mice were ether-anesthetized, the vena cavae exteriorized, and blood samples were obtained via a syringe fitted with a 25-gauge needle. Blood aliquots were allowed to clot at 22°C for 1 hour, and the sera removed and pipetted into a clean
25 receptacle. Total serum calcium was determined for each animal using a calibrated Vitros DT60 II analyzer.

Serum calcium data were plotted and pharmacokinetic parameters determined via

curve-fitting techniques using SigmaPlot software (Version 4.1). Means and standard deviations (or standard errors) were calculated and plotted to determine effect differences among dosing groups. Average serum calcium data for various conjugates are provided in Table 5 below.

5

Table 5

Conjugate	Dispersity	% Baseline Calcium Drop at 2.0 µg/kg dose
PEG7-Octyl-sCT, diconjugate	Monodispersed mixture	21.0
Stearate-PEG6-sCT, diconjugate	Monodispersed mixture	16.0
PEG7-Decyl-sCT, monoconjugate	Monodispersed mixture	11.5
Stearate-PEG8-sCT, diconjugate	Monodispersed mixture	11.0
PEG7-Decyl-sCT, diconjugate	Monodispersed mixture	8.3

Despite an *in vitro* activity as determined in Example 50 above that may not be comparable with the *in vitro* activity of PEG7-octyl-sCT and PEG7-decyl-sCT mono- and di-conjugates, the stearate-PEG6-sCT, diconjugate, and stearate-PEG8-sCT, diconjugate, appear to have *in vivo* activity (as evidenced by the drops in % baseline calcium from **Table 5** above) that are comparable with the *in vivo* activity observed for the PEG7-octyl-sCT and PEG7-decyl-sCT, mono- and di-conjugates. While not wanting to be bound by a particular theory, the improved *in vivo* activity of the stearate containing conjugates may indicate that these conjugates are undergoing hydrolysis *in vivo* to provide an active salmon calcitonin or active salmon calcitonin-PEG conjugate.

15

Example 131

The assay is as follows:

Cell culture: Stable clones expressing the full length human GHR were generated in 293 cells (human kidney embryonal cell line), designated 293GHR, as previously described.

20

Transcription assays: These were performed in 293 GHR cells transiently transfected with a reported construct containing a Stat5-binding element (LHRE) fused to a minimal TK promoter and luciferase. A β -galactosidase expression vector was cotransfected as a transfection control and luciferase values corrected for β -galactosidase activity. Sixteen hours after transfection, cells were transferred into serum free medium and treated with GH

or agonist for 6 hours. Luciferase activity is reported as percentage of maximal activity stimulated by GH in the specific experiment to allow comparison between repeated experiments. The maximal activity stimulated by GH is the fold induction stimulated by GH, i.e. corrected luciferase value in GH stimulated cells divided by corrected luciferase value in unstimulated cells. Results of the assay are shown in **Figures 42** and **43** where Genotropin is human growth hormone (standard, not part of the present invention), GH-002 is a 2 equivalent mTEG conjugate, GH-003 is a 5 equivalent mTEG conjugate, GH-004 is a 5 equivalent mTEG conjugate , Prot hGH is human growth hormone (standard, not part of the present invention), and hGH-TEG is a 9 equivalent mTEG conjugate.

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In the specification, there has been disclosed typical preferred embodiments of the invention and, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation, the scope of the invention being set forth in the following claims.

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